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Conservation status, range extension, and call analysis of the Littoral Glassfrog, *Cochranella litoralis* (Ruiz-Carranza and Lynch 1996)

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Abstract.—The little-known glassfrog *Cochranella litoralis* (Ruiz-Carranza and Lynch 1996) is a Vulnerable (VU) species infrequently reported in the literature. Its purported distribution includes the departments of Cauca and Nariño, Colombia, and the provinces of Esmeraldas, Los Ríos, Pichincha, and Santo Domingo de los Tsáchilas, Ecuador. Due to conflicting details regarding its distribution within the literature, we review past records to clarify which localities are valid. We also report two new localities that expand its elevational range to ≤ 407 m and its distribution approximately 175 km south from the previous southernmost locality, present an updated distribution map, and recommend an IUCN Red List status of Endangered (EN) for *C. litoralis*. Lastly, the call of *C. litoralis* is described for the first time, as is that of an Ecuadorian specimen of the widely-distributed *C. granulosa*.

Keywords. Amphibian, Anura, distribution, Ecuador, Endangered, threatened

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Resumen.—La poco conocida rana de cristal *Cochranella litoralis* (Ruiz-Carranza y Lynch 1996) es una especie Vulnerable (VU) con pocos registros en la literatura. Su distribución conocida incluye los departamentos de Cauca y Nariño, en Colombia, y las provincias de Esmeraldas, Los Ríos, Pichincha y Santo Domingo de los Tsáchilas, en Ecuador. Debido a que varias fuentes tienen detalles contradictorios con respecto a su distribución, revisamos los registros para mayor claridad, reportamos dos nuevas localidades que amplían su rango de altitud a ≤ 407 m y su distribución aproximadamente 175 km al sur de la localidad más al sur conocida, presentamos un mapa de distribución actualizado, y recomendamos que el estado de la Lista Roja de la UICN de *C. litoralis* se modifique a En peligro (EN). Por último, se describe por primera vez la llamada de *C. litoralis*, así como la de un ejemplar ecuatoriano de la ampliamente distribuida *C. granulosa*.

Palabras Claves. Anfibio, Anura, distribución, En peligro, amenazada

Introduction

The glassfrog genus *Cochranella* was first proposed over 70 years ago and included 13 species at that time (Taylor 1951). More recently, the genus was revised to resolve its former polyphyly, which reduced its membership to seven taxa (Guayasamin et al. 2009). Five species originally assigned to *Cochranella* were retained as *incertae sedis* within Centroleninae (i.e., “*Cochranella*”) due to a lack of molecular data and ambiguous behavioral and morphological characters (“*C.*” *balionota*, “*C.*” *duidaeana*, “*C.*”

megista, “*C.*” *riveroi*, “*C.*” *xanthocheridia*; Guayasamin et al. 2009). Two of the latter species have since been shown to belong to the genus *Nymphargus* (Guayasamin et al. 2019; Trageser et al. 2021). Currently, eight species are recognized within *Cochranella* (Frost 2024), as well as two putative new species (Guayasamin et al. 2020). Among the lesser known members is the threatened Littoral Glassfrog, *C. litoralis* (Ruiz-Carranza and Lynch 1996). For this species, relatively few observations have been reported, the call and tadpole have yet to be described, and the evolutionary relationships among its congeners are still

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uncertain (Twomey et al. 2014; Guayasamin et al. 2020).

The reported distribution of *C. litoralis* is restricted to lowland Chocoan rainforest below 250 m elevation in extreme southwestern Colombia and northwestern Ecuador (Ruiz-Carranza and Lynch 1996; Ruiz-Carranza et al. 1996; Grant and Morales 2010; IUCN SSC Amphibian Specialist Group 2019; Guayasamin et al. 2020). However, details in the literature regarding the extent of its distribution are ambiguous. While legitimate records have been reported from Nariño Department, Colombia, and Esmeraldas Province, Ecuador (Ruiz-Carranza and Lynch 1996; Guayasamin et al. 2006; Guayasamin et al. 2020; Pinto-Erazo et al. 2020), its distribution is also suggested to include Cauca Department, Colombia, and the Ecuadorian provinces of Los Ríos, Pichincha, and Santo Domingo de los Tsáchilas (Acosta-Galvis 2000; Lynch and Suárez-Mayorga 2004; IUCN SSC Amphibian Specialist Group 2019; Guayasamin et al. 2020). The latter references appear to have either conflicting data therein or lack corroborating material, or both. As a result, the extent of its known distribution is unclear.

Here, the literature and available material for *C. litoralis* is reviewed to clarify its known distribution and produce an updated distribution map that reflects verifiable localities. In addition, two new localities are reported that extend its known distribution 175 km south-southeast and mark the highest documented elevation, its call is described for the first time, and its extinction risk is reassessed. Lastly, the call of *C. granulosa* is described from a recently documented population in Ecuador (Culebras et al. 2020), as available call analyses of this taxon are based on populations from Costa Rica and Panama (Ibáñez et al. 1999; Kubicki 2007).

Materials and Methods

Field work was conducted at two separate sites. The first site was Los Laureles, Cotopaxi Province, Ecuador, where sampling efforts were conducted in March 2017 and March 2019. This area is characterized by a mosaic of cleared plots of land for agriculture and human settlements, with relatively small pockets of secondary forest. The second site was a fragmented forest near Cristobal Colón Quinde, Esmeraldas Province, Ecuador, where sampling was conducted in August 2021. The habitat consists of ca. 1.4 km² of secondary forest, with the northern end adjoining the Río Canandé. Large forest clearings are present to the east, south, and west. Patches of cleared forest are also present north of the Río Canandé, although intact mature forest is more prominent in this area and the protected forest of Reserva Biológica Río Canandé lies only about 3 km to the northwest, and Estación Biológica Jevon is just to the northeast of the forest fragment. The plot of forest sampled was recently purchased to serve as a future rescue center and sanctuary for the Critically Endangered Ecuadorian Brown-headed

Spider Monkey (*Ateles f. fusciceps*), and sampling of the biodiversity was conducted to generate a preliminary list of the taxa present in the forest.

Sampling was conducted using visual encounter surveys along trails and streams located within mature forest, disturbed forest, forest edge, and adjacent cleared areas, as well as agricultural plots. A Garmin 64s GPS receiver using WGS84 datum was used to collect geographic coordinates. Animals were verified as *Cochranella litoralis* using the diagnostic characters described in Ruiz-Carranza and Lynch (1996) and Guayasamin et al. (2020). Diagnostic photographs were taken of live specimens and submitted as vouchers to the digital repository at Centro Jambatu de Investigación y Conservación de Anfibios, San Rafael, Ecuador (CJ). Animals were returned to the exact location of capture after image and data collection, and released either the same night of capture or immediately at sunset the following evening to minimize stress. Field work was conducted under permit numbers 0013-18 IC-FAU-DNB/MA and MAE-ARSFC-2019-0163, authorized by the Ministerio del Ambiente del Ecuador, and carried out in accordance with the guidelines for the use of live amphibians and reptiles in field and lab research (Beaupre et al. 2004) compiled by the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, and the Society for the Study of Amphibians and Reptiles.

To assess and validate past records, we performed a search of the literature pertaining to *C. litoralis* as well as various databases containing unpublished specimens or locality information. The literature search was conducted by entering key words from its taxonomic history into Google Scholar (i.e., “*Centrolene litoralis*,” “*Centrolene litorale*,” and “*Cochranella litoralis*”). Public-sourced and museum databases that were assessed include: Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ; <https://bioweb.bio/faunaweb/amphibiaweb/>); Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN; <http://www.biovirtual.unal.edu.co/en/collections/search/amphibians/>); VertNet (<https://portal.vertnet.org/>); CalPhotos (<https://calphotos.berkeley.edu/>); iNaturalist (<https://www.inaturalist.org/>); and HerpMapper (<https://www.herpMapper.org/>). Confirmed localities were considered those that included a referenced specimen(s) or a combination of geographic coordinates and corroborating media. Extinction risk was assessed using the IUCN (2012) guidelines. Estimates for extent of occurrence (EOO) and area of occupancy (AOO) were calculated using the software GeoCAT (Bachman et al. 2011), following IUCN guidelines (IUCN 2022).

Bioacoustics. Call recordings for *C. litoralis* and *C. granulosa* were accessioned in the digital repository at Centro Jambatu de Investigación y Conservación de Anfibios, San Rafael, Ecuador (CJ). Call analyses for *C.*

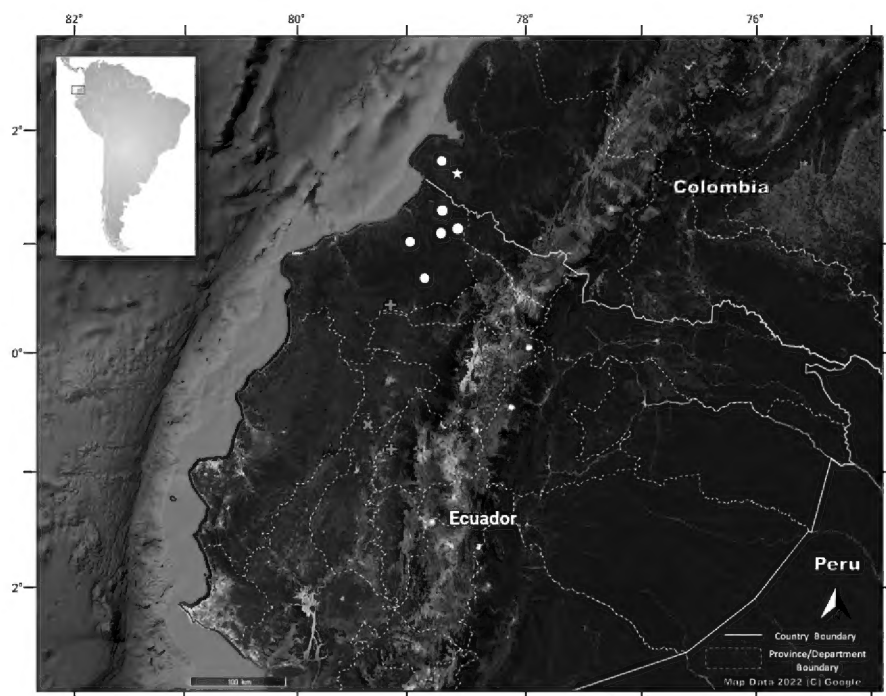


Fig. 1. Distribution map of *Cochranella litoralis*. White circles indicate verifiable localities reported in the literature; the white star denotes the type locality; the blue X marks the iNaturalist observation from the Río Palenque Research Center, Los Ríos; and yellow plus symbols indicate new records reported herein.

litoralis are based on four recordings of a single male (voucher CJ12588; call records: ec.cj.aud.26, ec.cj.aud.28–30) obtained by JC on 16 March 2019 between 0400–0430 h after light rain. The recordings were taken at Los Laureles, Cotopaxi, Ecuador within an abandoned banana plantation next to a small patch of secondary forest. That area frequently floods after rains and has a small, shallow creek with slow moving water. One recording was made with an iPhone 7 in MPEG-4 format with a sampling rate of 44.1 kHz and 24-bits resolution. The other three were made with a Tascam DR-05 recorder in WAV format with a sampling rate of 44.1 kHz and 16-bits resolution. The iPhone 7 was placed approximately 3.5 m from the calling male and the Tascam DR-05 recorder was placed less than 0.5 m away.

The call analysis of *C. granulosa* is based on seven recordings (ec.cj.aud.27, ec.cj.aud.31–36) taken from two males obtained by JC on 16 March 2019 between 0315–0340 h after light rain, and on 17 March 2019 between 2100–2115 h after light rain. The recordings were taken at Los Laureles, Cotopaxi, Ecuador, as reported by Culebras et al. (2020). The recordings were made with a Tascam DR-05 recorder in WAV format with a sampling rate of 44.1 kHz and 16-bits resolution. Recordings were made approximately 5 m from the calling male.

The Avisoft-SASLab Pro “Spectrogram tool” was used to analyze and filter the audio recordings. High resolution waveform, spectrogram, and power spectrum figures were generated using the R package Seewave (v2.2.1; Suer et al. 2008). To remove the background noise and facilitate the measurement of temporal and spectral parameters, a “Band Filter” was applied between 3,000–6,000 Hz and a “Noise Reduction” of 60 dB, with a threshold of -60 dB. The measurements were generated using Kaleidoscope Pro 5 software with the “Analyze View” tool, with a spectrogram configuration window

of a 512-sample window size and 512 FFT size. The parameters assessed, as defined by Köhler et al. (2017), were dominant frequency (frequency with the most energy), bandwidth (difference between the upper and lower frequencies) and call duration (length of a note).

Results

Cochranella litoralis (Ruiz-Carranza and Lynch, 1996)

New record. Adult male from Los Laureles, Cotopaxi, Ecuador (0°51'18.2232" S, 79°11'25.926" W, 407 m; Fig. 1), 16 March 2019 at 0400 h; Christophe Pellet and Jaime Culebras leg.; photo voucher CJ12588 (Fig. 2); uncollected. The specimen was observed calling shortly after a light rain, perched on a leaf 4.5 m high within an abandoned banana plantation adjacent to secondary forest. Other males have been observed at this same location, the first being on 19 February 2017 at 2100 h. Males of *Hyalinobatrachium tatayoi* have also been observed calling nearby.

New record. Adult male, 20.6 mm snout-urostyle length (SUL), recorded from a fragmented forest adjacent to Cristobal Colón Quininde, Esmeraldas, Ecuador (0.45213°N, 79.14919°W, 178 m; Fig. 1); 21 August 2021 at 2310 h; Ross Maynard and Sebastian Kohn leg.; photo vouchers CJ12587a–d (Fig. 2); uncollected. The male was observed in a clearing 3 m from the forest edge, calling on the upper surface of a leaf within sparse herbaceous vegetation, perched 1.0 m high. Slow, shallow water was channeled just below the vegetation due to steady rain earlier that evening, which was flowing towards a small stream (about 2–3 m wide and 0.5 m deep) a few meters away. Two additional males were heard calling nearby, one from just within the forest and the other also in the clearing near the forest edge, however their exact locations were not observed. Other glassfrogs recorded along the stream adjacent to where the *C. litoralis* was observed, but from within the secondary forest, were *Sachatamia ilex* and *Teratohyla spinosa*.

Distribution. A review of the literature yielded seven verified localities for *C. litoralis*: two localities from Colombia in extreme southwest Nariño Department, and five localities in Esmeraldas Province, Ecuador (Table 1; Fig. 1). The purported localities in Cauca, Colombia, and in Los Ríos, Pichincha, and Santo Domingo de los Tsáchilas, Ecuador, are either unverified or were reported in error (see **Discussion**). Nonetheless, a search of public-sourced and museum databases identified an additional locality from Los Ríos Province, Ecuador, at the Río Palenque Research Center in August 2021 (<http://iNaturalist.org/observations/90596035>; D. Weaver and E. Osterman, pers. comm.). Since the coordinates of the holotype provided by Ruiz-Carranza and Lynch (1996) are imprecise, the placement of the type locality on the map is approximated (Fig. 1). The new record from Los Laureles, Cotopaxi,

Table 1. Localities of *Cochranella litoralis* that are verifiable from deposited specimen(s), geographic coordinates, or corroborating media. PUCE = Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (<https://bioweb.bio/faunaweb/amphibiaweb/>); ** denotes an elevation that was acquired from Google Earth based on the coordinates provided.

	Locality / Coordinates	Specimen / Voucher #	Year(s) observed	Source
	Colombia, Nariño, Tumaco, La Guayacana / 1°49.8'N, 78°46.2'W, 100 m	ICN 13821	1985	Ruiz-Carranza and Lynch 1996
	Colombia, Nariño, Tumaco, Universidad Nacional de Colombia, Sede Tumaco / 1°36'22.1"N, 78°43'48.16"W, 17 m **	--	2015, 2016, 2020	Pinto-Eraza et al. 2020; iNaturalist.org
	Ecuador, Esmeraldas, Tsejpu, Río Zapallo / 0°42'0"N, 78°54'0"W, 150 m	EcoC 141	?	Grant and Morales 2004; Cisneros-Heredia and McDiarmid 2007
	Ecuador, Esmeraldas, Río Cachabí, 2 km NE on San Lorenzo-Lita Rd / 1°01'59.9" N, 78°46'0" W, 200 m	DHMECN 3198	2005	Guayasamin et al. 2006
	Ecuador, Esmeraldas, stream near Durango / 1°2'49.2"N, 78°37'4.8"W, 220 m	QCAZ 27693	2004	PUCE; Guayasamin et al. 2020
	Ecuador, Esmeraldas, Pichiyacu, Comunidad Chachi, Río Cayapas / 0°54'29.16"N, 78°59'52.8"W, 200 m	QCAZ 31705	1996	PUCE; Guayasamin et al. 2020
	Ecuador, Esmeraldas, Tundaloma Lodge / 1°10'43.248"N, 78°44'58.92"W, 74 m	QCAZ 57041; MZUTI 3481	2014	PUCE; Guayasamin et al. 2020
	Ecuador, Los Ríos, Centro Científico Río Palenque / 0°35'17.47"S, 79°21'44.95"W, 170 m	--	2021	iNaturalist.org
	Ecuador, Esmeraldas, Cristobal Colón Quininde / 0°27'7.668"N, 79°8'57.084"W, 178 m	CJ12587a-d	2021	This study
	Ecuador, Cotopaxi, Los Laureles / 0°51'18.2232"S, 79°11'25.926"W, 407 m	CJ12588	2017, 2019	This study

expands the elevational range from near sea level to 407 m asl, and extends the known distribution of *C. litoralis* by about 175 km south-southeast from the previous southernmost locality at Tsejpu, Río Zapallo, Esmeraldas.

Extinction risk. Despite the new records, the extinction risk for *C. litoralis* remains relatively high. With the additional localities reported herein, and assuming each of the seven localities where the species had previously been reported represent extant populations, the extent of occurrence (EOO) of the species is about 8,308 km² and the area of occupancy (AOO) is 40 km². However, the only other reported observations over the past decade are from Tundaloma Lodge, Esmeraldas, Ecuador in 2014 (Guayasamin et al. 2020) and Tumaco, Nariño, Colombia, in 2015, 2016, and 2020 (Table 1; IUCN SSC Amphibian Specialist Group 2019; Pinto-Erazo et al. 2020; iNaturalist.org). Except for the latter locality in Colombia, whether there have been subsequent sampling efforts for *C. litoralis* at the remaining localities in Esmeraldas Province, Ecuador is unclear. Although the status of these subpopulations cannot be verified at this time, we suspect that there has been recent and ongoing decline in the extent and quality of its habitat, given that northwest Ecuador has been a hotspot of deforestation over the past three decades (Sierra 2013; Kleeman et al. 2022). Logging and agriculture are

the main drivers of deforestation in the region, which have resulted in severely fragmented forests throughout its range. As a result of these ongoing pressures, *C. litoralis* is currently known only from threat-defined locations (*sensu* IUCN 2012, 2022). While the observations reported here are the first to suggest that the species can tolerate altered habitat adjacent to forest, at least to some degree, the natural history and habitat requirements of the species remain poorly understood. Accordingly, and like the recent threat assessment for its national status in Ecuador (Ortega-Andrade et al. 2021), we recommend a global threat status of Endangered (EN) for *C. litoralis* following IUCN criteria B2ab(iii).

Call analysis. The call of *C. litoralis* consists of a short, single tonal note (Fig. 3). The call duration was 88.51–177.17 ms ($\bar{x} = 132.84 \pm 44.33$; $N = 4$), the dominant frequency ranged from 5,210–5,304 Hz ($\bar{x} = 5,257 \pm 47$; $N = 4$), and the call bandwidth ranged from 738–1,729 Hz ($\bar{x} = 1,265 \pm 527$; $N = 4$).

Compared to the available call descriptions of other species in the genus, *C. nola* and *C. mache* have similar call structures and parameter metrics. While *C. nola* exhibits a simple, non-pulsed note with comparable metrics (call duration: $\bar{x} = 95$ ms ± 11.97 , dominant frequency: $\bar{x} = 5,460$ Hz ± 221 ; Lötters and Köhler 2000; Köhler et al.

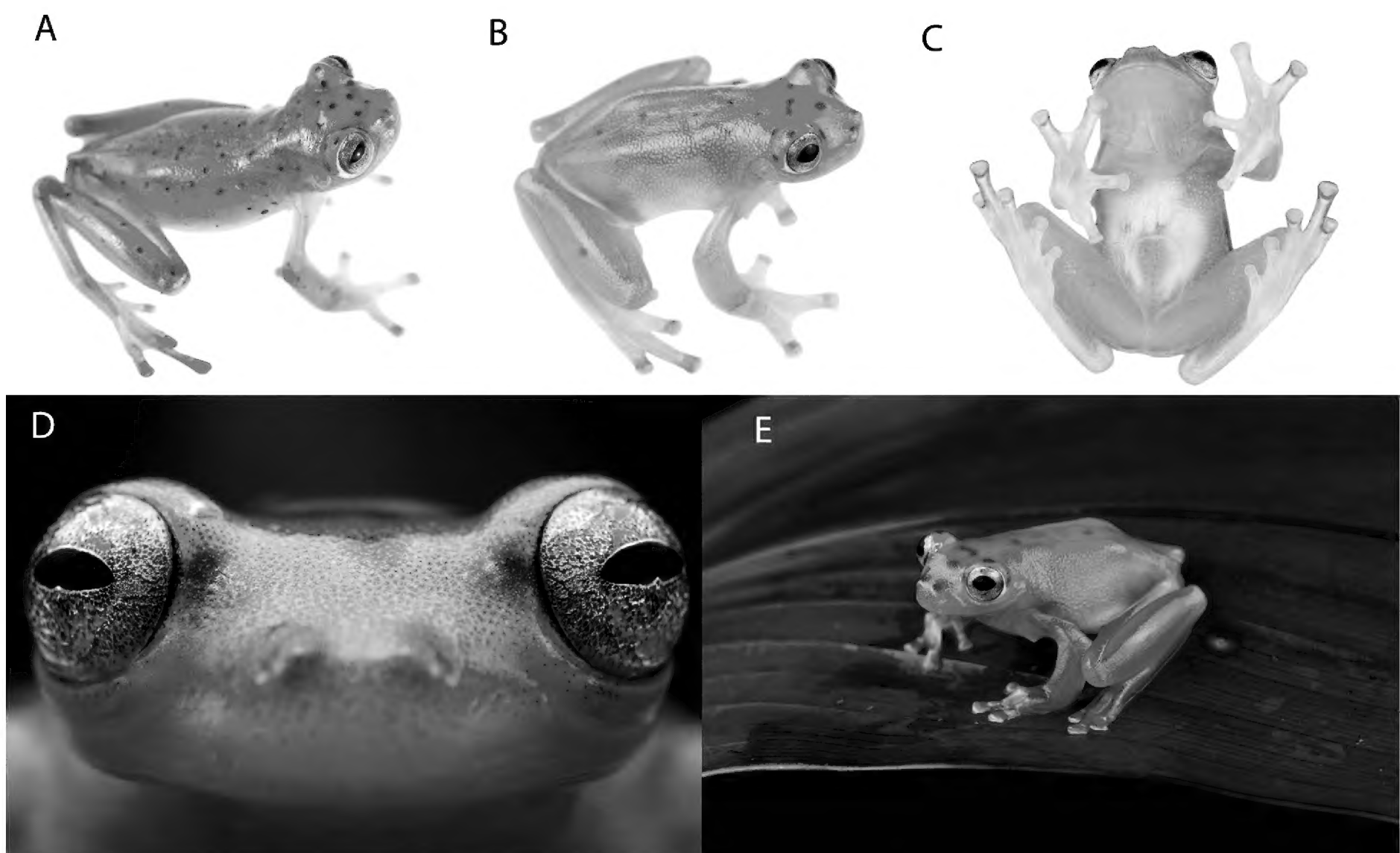


Fig. 2. Dorsal and ventral aspects of *Cochranella litoralis* in life. (A) Adult male, CJ12588, from Los Laureles, Cotopaxi, Ecuador. (B–E) Adult male, CJ12587a–d, from Cristobal Colón Quininde, Esmeraldas, Ecuador. Photos by: Jaime Culebras (A); Ross J. Maynard (B–E).

Call analysis and conservation of *Cochranella litoralis*

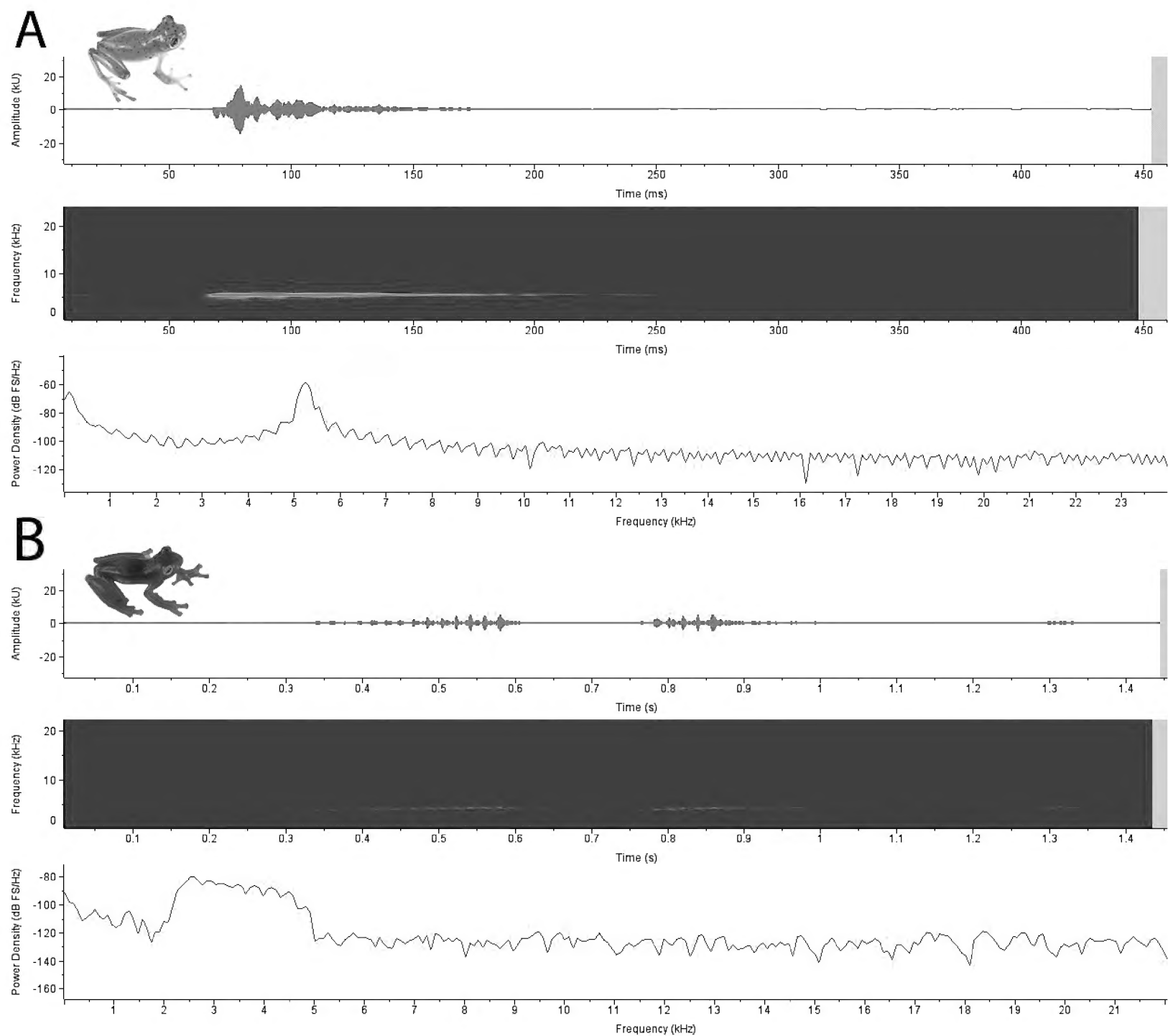


Fig. 3. Audio spectrogram (top), oscillogram (middle), and power spectrum (bottom) of an adult male *Cochranella litoralis* from Los Laureles, Cotopaxi, Ecuador (A), and an adult male *C. granulosa* from Jardín de los Sueños, Cotopaxi, Ecuador (B).

2006), *C. mache* has a call with two pulsed notes, a call duration of $\bar{x} = 38 \text{ ms} \pm 8$, and a dominant frequency of $\bar{x} = 5,410.2 \text{ Hz} \pm 17.9$ (Ortega-Andrade et al. 2013). Other *Cochranella* spp. that have described calls, such as *C. granulosa* (Fig. 3), have pulsed notes.

The call metrics measured from seven call recordings of *C. granulosa* observed at Jardín de los Sueños, Cotopaxi, Ecuador are as follows. Calls consisted of 1–4 notes ($\bar{x} = 2.34$), with 8–15 pulses per note; the first notes in multi-note calls are more pulsed than subsequent notes (first note: $\bar{x} = 15 \pm 2$ pulses; second note: $\bar{x} = 12 \pm 2$ pulses; third note $\bar{x} = 8 \pm 5$ pulses); call duration was 150–1,437 ms ($\bar{x} = 790 \text{ ms} \pm 640$); single-note duration varied from 130–260 ms, with the first note generally being longer than subsequent notes, similar to the calls of individuals from Costa Rica (Ibáñez et al. 1999; Kubicki 2007). The note interval was 45.49–179.31 ms ($\bar{x} = 112 \pm 66.91$), and the dominant frequency was measured at 3,943–4,119 Hz ($\bar{x} = 4,031 \pm 88$). Comparable metrics can be found in *C. guayasamini*, as it also exhibits a high-pitched, pulsed trill with two notes, with the first note having substantially more pulses than the second note (Twomey

et al. 2014). Similar to the lack of phenotypic variation observed between populations of *C. granulosa* in Ecuador and Central America (Culebras et al. 2020), call variation also appears to be minimal.

Discussion

Although information for *C. litoralis* remains limited, this study contributes new locality records and the first call analysis of this species, as well as that of *C. granulosa* from Ecuador. Our observation from Los Laureles, Cotopaxi, and the observation identified in the iNaturalist database from the Río Palenque Research Center, Los Ríos, are the first verified records outside of either Nariño Department, Colombia, or Esmeraldas Province, Ecuador. Other works that suggest its distribution includes Cauca Department, Colombia, and the Ecuadorian provinces of Pichincha, Santo Domingo de los Tsáchilas, and Los Ríos seem to do so in error or cannot be confirmed. Acosta-Galvis (2000) was the first to report the species from Cauca, but this was a mistake when citing the original description by Ruiz-Carranza and Lynch (1996). Lynch and Suárez-Mayorga (2004) inexplicably report Guapi, Cauca as the species'

only locality in Colombia, while omitting the type locality of La Guayacana, Nariño. Considering that there appears to be no evidence to support that locality, we suspect the former error led to the latter. The most recent Red List assessment for *C. litoralis* also includes the Guapi locality, but the uncertainty of that locality is acknowledged (IUCN SSC Amphibian Specialist Group 2019). Notably, the only observations of *C. litoralis* from Colombia since it was described were reported from adjacent to the type locality in Nariño (Pinto-Eraza et al. 2020), whereas no localities have been reported from Cauca.

The available information for populations within Ecuador is also confusing. The Red List assessment states that *C. litoralis* is known from the provinces of Esmeraldas, Santo Domingo de los Tsáchilas, and Los Ríos, however, the range map and extent of occurrence (EOO) exclude the latter two provinces (IUCN SSC Amphibian Specialist Group 2019). Although it is unclear why these provinces are mentioned, Cisneros-Heredia and McDiarmid (2007) suggested that two specimens collected in 1979 from the Río Palenque Research Center, Los Ríos, represent an undescribed taxon that is morphologically similar to *C. litoralis*. That locality lies on the border of Los Ríos and Santo Domingo de los Tsáchilas, which may have led to the confusion. Cisneros-Heredia and McDiarmid (2007) posit that the Río Palenque specimens are distinguishable from *C. litoralis* based on a difference in iris coloration—unique red marks and reticulations as opposed to a salmon iris—but such variation is evident in images of *C. litoralis* throughout much of its known distribution, including from the vicinity of the Río Palenque Research Center (Fig. 2; iNaturalist.org; Cisneros-Heredia and McDiarmid 2007; Guayasamin et al. 2020). Therefore, we believe it is unlikely that the specimens from the Río Palenque Research Center are distinguishable from *C. litoralis*, and, if true, that the taxon has been observed at this site in January 1979 and August 2021 (<https://www.inaturalist.org/observations/90596035>; <https://collections.nmnh.si.edu/search/herps/>; Cisneros-Heredia and McDiarmid 2007).

Guayasamin et al. (2020) provide five localities with referenced vouchers and geographic coordinates for *C. litoralis* from Ecuador (see subsections “Specimens examined” and “Localities from the literature” therein). These data conflict with the localities depicted in the associated distribution map in both geographic position and number of localities, so we view these localities from the map as either unconfirmed or reported in error (Guayasamin et al. 2020). Guayasamin et al. (2020) also informally mentioned the presence of *C. litoralis* at Jardín de los Sueños, Cotopaxi, presumably based on an observation uploaded to the iNaturalist database by one of the authors of this paper (CP). However, and as we report herein, the observations from this area are not from the latter site, but instead from the nearby site of Los Laureles, Cotopaxi.

Considering that the conservation status of *C. litoralis* is primarily based on distribution data (IUCN

SSC Amphibian Specialist Group 2019), our review of past localities along with the new records reported here provides an updated basis from which its extinction risk can currently be assessed. Like prior reports of this species, our observations are not from within protected areas. Nonetheless, the observation from Los Laureles, Cotopaxi, was made about 2 km from the private reserve of Jardín de los Sueños, and is the second substantial range extension of a glassfrog discovered from the area (Culebras et al. 2020). Although the observation from Cristobal Colón Quinde, Esmeraldas, is only 3 km south of Reserva Biológica Río Canandé, there have surprisingly been no observations of *C. litoralis* documented there, although this area has been fairly well sampled (Mite et al. 2013).

Overall, the call analyses we provide improve our understanding of the natural history of these taxa, and can benefit efforts in field detection and studies of their respective species boundaries. Nonetheless, these glassfrogs remain poorly understood and there is little data to inform population trends either locally or across their known distributions. Future efforts are needed to fill these knowledge gaps, especially in light of the ongoing, broad-scale declines in the forest ecosystems from which they are known (Sierra 2013; Kleeman et al. 2022).

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Literature Cited

- Acosta-Galvis AR. 2000. Ranas, Salamandras y Caecilias (Tetrapoda: Amphibia) de Colombia. *Biota Colombiana* 1(3): 289–319.
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B. 2011. Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. In: Editors, Smith V, Penev L. e-Infrastructures for Data Publishing in Biodiversity Science. *Zookeys* 150: 117–126. (Version BETA).
- Beaupre SJ, Jacobson ER, Lillywhite HB, Zamudio K. 2004. *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research*. American Society of Ichthyologists and Herpetologists, Lawrence, Kansas, USA. 43 p.
- Cisneros-Heredia DF, McDiarmid RW. 2007. Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glassfrogs. *Zootaxa* 1572: 1–82.
- Culebras J, Angiolani-Larrea FN, Tinajero-Romero J, Pellet C, Yeager J. 2020. First record and notable range extension of the glass frog *Cochranella granulosa*

- (Taylor, 1949) (Anura, Centrolenidae) found in Ecuador. *Herpetology Notes* 13: 353–355.
- Frost DR. 2024. Amphibian Species of the World: an Online Reference. Version 6.1. Available: <https://amphibiansoftheworld.amnh.org/index.php>. [Accessed: 4 September 2022].
- Grant T, Morales M. 2010. *Cochranella litoralis*. The IUCN Red List of Threatened Species 2010: e.T54923A11225350.
- Guayasamin JM, Cisneros-Heredia DF, Yáñez-Muñoz MH, Bustamante MR. 2006. Notes on geographic distribution. Amphibia, Centrolenidae, *Centrolene ilex*, *Centrolene litorale*, *Centrolene medemi*, *Cochranella albomaculata*, *Cochranella ametarsia*. Range extensions and new country records. *Check List* 2: 24–25.
- Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilà C. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.
- Guayasamin JM, Cisneros-Heredia DF, McDiarmid RW, Peña P, Hutter CR. 2020. Glassfrogs of Ecuador: diversity, evolution, and conservation. *Diversity* 12(6): 222.
- Ibáñez DR, Rand AS, Jaramillo A. 1999. *Los Anfibios del Monumento Natural Barro Colorado, Parque Nacional Soberanía y Áreas Adyacentes*. Editorial Mizrahi y Pujol, Santa Fe de Bogotá, Colombia. 187 p.
- IUCN SSC Amphibian Specialist Group. 2019. *Cochranella litoralis*. The IUCN Red List of Threatened Species 2019: e.T54923A49367704.
- IUCN Standards and Petitions Committee. 2022. *Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.1*. Prepared by the Standards and Petitions Committee, International Union for the Conservation of Nature, Gland, Switzerland. 114 p.
- Kleeman J, Zamora C, Villacis-Chiluisa AB, Cueva P, Koo H, Noh JK, Fürst C, Thiel M. 2022. Deforestation in continental Ecuador with a focus on protected areas. *Land* 11(268): 1–26.
- Köhler J, John A, Böhme W. 2006. Notes on amphibians recently collected in the Yungas de La Paz region, Bolivia. *Salamandra* 42(1): 21–27.
- Köhler J, Jansen M, Rodriguez A, Kok PJ, Toledo LF, Emmrich, M, Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods, and recommendations for best practice. *Zootaxa* 4251(1): 1–124.
- Kubicki B. 2007. *Ranas de Vidrio de Costa Rica/Glass Frogs of Costa Rica*. Editorial INBio, Santo Domingo de Heredia, Costa Rica. 304 p.
- Lötters S, Köhler J. 2000. *Cochranella nola* (Anura: Centrolenidae): natural history notes, distribution, and advertisement call. *Herpetological Natural History* 7(1): 79–81.
- Lynch JD, Suarez-Mayorga A. 2004. Catálogo de anfibios en el Chocó Biogeográfico. Pp. 654–668 In: *Colombia Diversidad Biótica IV, El Chocó Biogeográfico/Costa Pacífica. Volumen I*. Editor, Orlando Rangel C. Universidad Nacional de Colombia, Bogotá, Colombia. 862 p.
- Mite MAM, Yáñez-Muñoz MH, Meza-Ramos PA, Reyes-Puig MA. 2013. Reserva Biológica Río Canandé. Entre los últimos relictos de bosque húmedo Tropical en la Costa. In: *Herpetofauna en Áreas Prioritarias para la Conservación: el Sistema de Reservas Jocotoco y Ecominga*. Serie de Publicaciones del Museo Ecuatoriano de Ciencias Naturales, Monografía 6. MECN, Fundación para la Conservación Jocotoco, Fundación Ecominga, Quito, Ecuador. 392 p.
- Ortega-Andrade HM, Rojas-Soto O, Paucar C. 2013. Novel data on the ecology of *Cochranella mache* (Anura: Centrolenidae) and the importance of protected areas for this Critically Endangered glassfrog in the Neotropics. *PLoS ONE* 8(12): e0081837.
- Ortega-Andrade HM, Blanco MR, Cisneros-Heredia DF, Arévalo NG, Vargas-Machuca KGL, Sánchez-Nivicela JC, Armijos-Ojeda D, Andrade JFC, Reyes-Puig C, Riera ABQ, et al. 2021. Red List assessment of amphibian species of Ecuador: a multidimensional approach for their conservation. *PLoS ONE* 16(5): e0251027.
- Pinto-Erazo MA, Calderón-Espinosa ML, Medina-Rangel GF, Galeano MAM. 2020. Herpetofauna de dos municipios del suroeste de Colombia. *Biota Colombiana* 21(1): 41–57.
- Ruiz-Carranza PM, Lynch JD. 1996. Ranas Centrolenidae de Colombia IX. Dos nuevas especies del suroeste de Colombia. *Lozania Acta Zoológica Colombiana* 30(68): 1–11.
- Ruiz-Carranza PM, Ardila-Robayo MC, Lynch JD. 1996. Lista actualizada de la fauna de Amphibia de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas, y Naturales* 20(77): 365–415.
- Sierra R. 2013. *Patrones y Factores de Deforestación en el Ecuador Continental, 1990–2010, y un Acercamiento a los Próximos 10 Años*. Conservación Internacional Ecuador / Forest Trends, Quito, Ecuador. 45 p.
- Suer J, Augin T, Simonis C. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18(2): 213–226.
- Taylor EH. 1951. Two new genera and a new family of tropical American frogs. *Proceedings of the Biological Society of Washington* 64: 33–40.
- Trageser SJ, Maynard RJ, Culebras J, Kohn S, Quezada A, Guayasamin JM. 2021. Phylogenetic position of the glassfrog “*Cochranella*” *megista* (Anura: Centrolenidae) and first records for Ecuador. *Phyllomedusa* 20: 27–35.
- Twomey EM, Delia J, Castroviejo-Fisher S. 2014. A review of northern Peruvian glassfrogs (Centrolenidae), with the description of four new remarkable species. *Zootaxa* 3851: 1–87.



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Evaluation of p-Chip microtransponder tags on small-bodied salamanders (*Eurycea* spp.)

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Abstract.—Reliable approaches for tracking individual organisms are needed for research purposes and to inform the conservation and management of aquatic organisms. However, safe and dependable tagging methods are difficult to implement for small-bodied organisms. The objective of this study was to examine survival, tag retention, and growth in three aquatic salamander species of different sizes (Barton Springs Salamander, *Eurycea sosorum*; Comal Springs Salamander, *Eurycea pterophila*; Texas Blind Salamander, *Eurycea rathbuni*) injected with p-Chip tags in a captive setting. The ability of novice scanners to read p-Chips over the duration of the study was also assessed. Post-tagging survival was high across all treatments for all species (97–100%). Tag retention among species was similar (97–100%), and growth appeared unaffected by tagging. No relationship between success of tag readability and time since tagging was found, and all novice scanners were able to read the tags implanted in 100% of Comal Springs and Texas Blind Salamanders. However, variability was found with novice scanners reading tags in Barton Springs Salamanders, although all tags were successfully read by an experienced scanner. P-Chips provided an improved readability rate, reduced human error, and allowed for more individual identification codes than the visible implant elastomer tags commonly used for these species. This study shows that p-Chips are suitable tags for small-bodied aquatic salamanders.

Keywords. *Eurycea sosorum*, *Eurycea pterophila*, *Eurycea rathbuni*, retention, survival, tracking

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Introduction

Wildlife tagging provides a reliable method for the identification and differentiation of individuals that would otherwise be challenging to distinguish, and it allows for tracking a variety of life history parameters (Ricker 1956). In the field, mark-recapture efforts can provide information about growth, survival, habitat range, migration, age, condition, and other key parameters (Ricker 1956; Silvy et al. 2005; Osbourn et al. 2011; Moon et al. 2022) and marked organisms can be monitored over time in individuals, cohorts, and subsets of populations and communities. In captive settings, tagging eliminates the need to separate organisms or cohorts into different tanks or enclosures for identification, allowing enclosures to be stocked to capacity, thereby conserving space. It also facilitates the tracking of rare occurrences in captive individuals, such as reproductive events or illness. However, tags

that are not compatible with a species can cause injury, mortality, or behavioral changes in the organism due to stress and difficulty in functioning normally (Musselman et al. 2017; Moon et al. 2022). In these cases, the results of any tagging program would be negatively affected by the tagging method.

A long and growing list of wildlife marking methods exists, allowing researchers to tailor methods to their study needs while accounting for the organism's biology and life history (Silvy et al. 2005). Tags should be selected to maximize various factors such as tag retention and longevity, cost efficiency, and ease of use while minimizing stress, handling time, and other factors that negatively affect growth, behavior, and survival (Osbourn et al. 2011). Failure to mitigate these negative effects can violate mark-recapture assumptions (Ricker 1956) and tagging ethics (Cooke et al. 2013), preventing the extrapolation of meaningful conclusions from data (Murray and Fuller 2000). General amphibian

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tagging practices include digit clipping (Phillott et al. 2007; Waddle et al. 2008), branding (Donnelly et al. 1994; Measey et al. 2001), affixing or implanting radio transmitters (Weick et al. 2005), staining (Carlson and Langkilde 2013; Fischer et al. 2020), pattern mapping and software assisted photo identification (Andreone 1986; Gamble et al. 2008; Bendik et al. 2021), injecting elastomers, and attaching transponders (Sinsch 1997; Donnelly et al. 1994; Moon et al. 2022).

Central Texas is populated by several species of endemic, paedomorphic salamanders of state and federal conservation concern with narrow distributions and poorly understood demographics. Among these are the state and federally endangered Texas Blind Salamander (*Eurycea rathbuni*) and Barton Springs Salamander (*Eurycea sosorum*), and a population of the non-listed Fern Bank Salamander from Comal Springs (reclassified from *Eurycea neotenes* to *Eurycea pterophila* by Devitt et al. 2019). The Barton Springs Salamander is protected under the Barton Springs Habitat Conservation Plan (Barton Springs/Edwards Aquifer Conservation District 2018), and the Texas Blind Salamander and Comal Springs Salamander are protected under the Edwards Aquifer Habitat Conservation Plan (Edwards Aquifer Authority 2012). Tagging these animals requires accommodating their small size and permeable skin (Heemeyer et al. 2007). This is achieved by selecting small tags with a high tag retention rate associated with that species. Additionally, it is important to minimize mortality when working with threatened and endangered species, so stress and handling time should be considered.

Few studies have used tagging techniques specifically on either Barton Springs, Texas Blind, or Comal Springs Salamanders. Bendik et al. (2021) successfully employed photographic identification software to track Barton Springs Salamanders without placing a tag. However, when using photo identification in Jollyville Plateau Salamanders (*Eurycea tonkawae*), another paedomorphic salamander species found in Central Texas, misidentification rates slowly increased over time in adults and rapidly increased (within 2 months) in juveniles due to growth-associated pigment changes (Bendik et al. 2013). Passive Integrated Transponder (PIT) tags, bio-compatible glass-encased microchips typically ranging in size from 8–14 mm (Gibbons et al. 2004), yielded a low retention rate in adult Texas Blind Salamanders (Moon et al. 2022). PIT tags are best suited for larger individuals and species over 50 mm in standard length (Musselman et al. 2017), rendering them unsuitable for juveniles and smaller species. Visible Implant Alphanumeric (VIA) tags, small (1.2 mm x 2.7 mm to 2.0 x 5.0 mm) fluorescent plastic tags printed with visible 3-digit alphanumeric codes (Northwest Marine Technology Inc. 2019), were rejected by Comal Springs Salamanders by tearing through the skin and falling out through the resulting wound (Moon et al.

2022). Even when salamanders successfully retained VIA tags (e.g., Texas Blind Salamanders; Moon et al. 2022), reading was inhibited when the tag was injected too deeply beneath an insufficiently translucent tissue (Osbourn et al. 2011), or at an angle to the epidermal surface (Moon et al. 2022). Visible Implant Elastomer (VIE) tags, injectable colored liquid tags that cure into flexible, fluorescent, bio-compatible solids (Northwest Marine Technology Inc. 2019), exhibited great ease of use and retention in Texas Blind and Comal Springs Salamanders (Moon et al. 2022) and had no observable effects on survival or growth in San Marcos salamanders (*Eurycea nana*) (Phillips and Fries 2009). VIE tags are injected with a 29-gauge needle (Davis and Ovaska 2001), making them suitable for small salamanders. Tag reading success was generally high (Moon et al. 2022), but has been reported to be reduced in some cases by the propensity of the tags to break, degrade, and migrate (Heemeyer et al. 2007). Additionally, certain elastomer colors are difficult to discern even by a trained eye (DMM, TSF, pers. obs.; Northwest Marine Technology Inc. 2021), occasionally resulting in human error. As a result, the sparse color palette available limits the number of differentiable markings possible (≤ 10) unless using multiple color injections, which increases handling time (Davis and Ovaska 2001), requires additional wounds, and exacerbates stress.

As a relatively new technology, p-Chips are injectable microtransponders with a unique set of characteristics that present an alternative option for tagging small-bodied species, including neotenic salamanders. Tag detection and reading is accomplished with a laser wand that transmits information in the form of a 9-digit serial number from the p-Chip photocells to a computer using specialized software (PharmaSeq, Princeton, New Jersey, USA), which expedites tag detections and readings and eliminates the potential for human error. The small size of p-Chips (500 μm x 500 μm x 100 μm) renders them nearly invisible and should not provoke social (Fiske 1997; Frommen et al. 2015; Fischer et al. 2020) or predatory (Catalano et al. 2001; Carlson and Langkilde 2013) behavioral responses from surrounding animals, which are sometimes associated with colored tags. P-Chips have shown high tag retention (Chen et al. 2013; Moore and Brewer 2021) and subject survival rates (Faggion et al. 2020; Moore and Brewer 2021) in studies performed on small-bodied fishes. Thus, p-Chips could be effective for other small-bodied aquatic species, including salamanders.

To our knowledge, p-Chip microtransponder tags have not yet been tested in salamanders or any other amphibians. The purpose of this study was to examine the efficacy of p-Chips in small-bodied aquatic salamanders by measuring survival and tag retention in three salamander species of different sizes (*Eurycea sosorum*, *Eurycea pterophila*, and *Eurycea rathbuni*) injected with p-Chip tags in a captive setting.

Materials and Methods

All salamanders used in this study, namely Barton Springs Salamanders (*Eurycea sosorum*, $n = 95$), Comal Springs Salamanders (*Euryceapterophila*, $n = 111$), and Texas Blind Salamanders (*Eurycea rathbuni*, $n = 78$), were part of the captive-assurance populations (i.e., Critically Endangered and threatened animals in captivity for reintroduction purposes) located at the United States Fish and Wildlife Service San Marcos Aquatic Resources Center in San Marcos, Texas, USA. When possible, we prioritized using captive-bred salamanders to minimize potential harm to the wild stock population. However, due to the limited availability of captive-bred individuals, 51 captive-held wild stock Texas Blind Salamanders were included. All salamanders in this study were adults except for 19 juvenile captive-bred Texas Blind Salamanders. Adult salamanders were held in seven tanks, each with a volume of approximately 265 L (70 gal), maintained at a depth of 23 cm. Each tank of adult salamanders contained one species of salamander and was divided into equal sections with water-permeable barriers to separate the treatment groups while maintaining controlled environmental conditions across the groups. Juvenile salamanders were held in three 38 L (10 gal) aquaria with an 18 cm water depth. Each aquarium contained one treatment group, but all aquaria received water from the same source to keep the water quality consistent. Tanks were supplied with flow-through well water at a temperature of 20–23 °C. Each tank section and aquarium had a similar assortment of habitat structures (rocks, aquarium plants, etc.). Adult salamanders were fed live blackworms and live *Daphnia* once weekly, and live *Daphnia* and frozen *Mysis* once weekly. Additionally, because of their larger size, adult Texas Blind Salamanders were fed live red worms (*Eisenia fetida*, *Eisenia hortensis*, and *Perionyx excavatus*) cut into small pieces each week. Juvenile Texas Blind Salamanders were fed live *Artemia* and *Daphnia* twice weekly. All feeds except for the red worms were supplied at a portion of 0.25 mL/salamander. Red worms were supplied at a portion of 1.6 cm/salamander. Tanks were cleaned weekly.

Prior to launching the full study, we ran a pilot study with five salamanders of each species tagged using the methods described below and monitored for one month to assess potential mortality in the federally listed species. All salamanders survived and retained their tags. Pilot study salamanders were not used in any analyses.

Salamanders were anesthetized before being placed into treatment groups, and measurements were taken. Salamanders were anesthetized via immersion in tricaine methanesulfonate (MS-222, 0.5 g/L) buffered with sodium bicarbonate using previously established protocols (Wright 2001). The salamanders were randomly assigned into treatment groups (tagged, sham, and control) using a random number generator. Sample size varied among treatments due to the limited availability of salamanders (Table 1). The tagged groups were the most numerous for

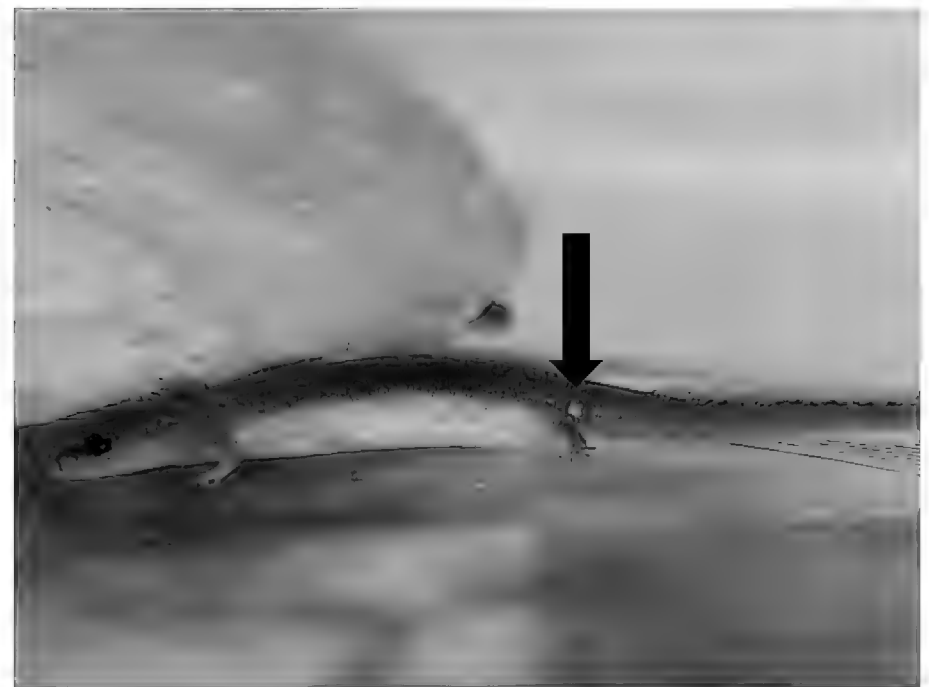


Fig. 1. The left side of a gravid female salamander. The black arrow points to the p-Chip tag.

each species to ensure the validity of statistical analyses examining tag retention. Salamanders were placed in clear re-sealable, sliding channel, polyethylene storage bags for easier handling (Heemeyer et al. 2007). Each salamander was measured for weight (g) and snout-vent length (SVL, mm; Petranks 1998; Table 1), sexed using the candling method (Gillette and Peterson 2001), and any distinguishing features or behaviors were recorded (e.g., the presence of eggs or regurgitation). Technical difficulties prohibited the weighing of 40 of the 95 Barton Springs Salamanders (Table 1).

Three treatment groups were used to examine the effects of p-Chips on salamander survival. Following the manufacturer's guidelines (Pharmaseq Inc. 2020), a 0.8 mm diameter hypodermic needle was used to inject a 500 μm x 500 μm x 100 μm p-Chip subcutaneously at the base of the tail just dorsal and posterior to the left hindlimb of each salamander in the tagged groups (Fig. 1). After tagging, the p-Chips were scanned with the laser reader to record the unique 9-digit tag number. Sham salamanders were treated the same as tagged salamanders (e.g., handled and punctured with the needle) except no tag was placed. Control salamanders were handled the same as the tagged and sham salamanders but were not tagged or pierced with a needle. Using both sham and control groups allowed us to distinguish between the effects of the handling process and the effects of the tag itself (Jepsen et al. 2015; Moore and Brewer 2021). After handling, the salamanders were placed in a small recovery tank until they were able to right themselves and swim normally. Salamanders were then moved to the appropriate section of their holding tank. For consistency, one researcher (DM) performed all the tagging.

Salamander survival, tag retention, and tag readability were monitored for eight months, and growth was examined at the conclusion of the study. Survival was monitored daily as part of the normal husbandry care. Each week, a researcher with experience in scanning p-Chips (DM) scanned all tagged salamanders to check

Table 1. Mean snout-vent lengths (SVL; \pm SD), weights (\pm SD), ranges, and sex (M = male, F = female, U = unknown) for each treatment at the start of the study. Only a subset of the Barton Springs Salamanders were weighed due to technical difficulties, and the number weighed is indicated in parentheses after the sample size (*n*). The final SVL was obtained at the conclusion of the study.

Treatment	<i>n</i>	Mean SVL (mm) \pm SD	SVL (mm) range	Mean weight (g) \pm SD	Weight (g) range	Final mean SVL (mm) \pm SD	Final SVL (mm) range	M	F	U
Barton Springs Salamander (<i>Eurycea sosorum</i>)										
Total	95 (55)	41.5 \pm 4.1	32–52	1.8 \pm 0.6	0.8–3.7	41.6 \pm 4.4	32–52	33	53	9
Control	32 (18)	40.8 \pm 3.5	35–47	1.5 \pm 0.4	0.8–2.4	40.9 \pm 3.8	33–47	14	14	4
Sham	31 (18)	41.1 \pm 4.6	32–48	1.8 \pm 0.6	1.1–3.6	41.0 \pm 4.8	32–51	17	11	3
P-Chip	32 (19)	42.4 \pm 4.0	36–52	2.0 \pm 0.7	1.1–3.7	42.7 \pm 4.4	35–52	22	8	2
Comal Springs Salamander (<i>Eurycea pterophila</i>)										
Total	111	32.9 \pm 2.2	29–39	0.7 \pm 0.1	0.4–1.1	32.4 \pm 1.8	28–38	67	44	0
Control	34	32.6 \pm 2.2	29–37	0.6 \pm 0.1	0.4–0.9	31.7 \pm 1.9	28–36	18	16	0
Sham	34	33.0 \pm 2.1	30–39	0.7 \pm 0.2	0.4–1.1	33.2 \pm 1.8	30–38	21	13	0
P-Chip	43	33.0 \pm 2.4	29–39	0.7 \pm 0.1	0.4–1.1	32.3 \pm 1.7	28–36	28	15	0
Texas Blind Salamander (<i>Eurycea rathbuni</i>)										
Total	78	49.4 \pm 11.0	26–67	3.3 \pm 1.8	0.35–8.1	50.5 \pm 10.2	28–68	33	18	27
Control	20	48.6 \pm 12.1	28–64	3.2 \pm 1.9	0.5–6.3	49.7 \pm 10.6	32–66	13	2	5
Sham	20	47.9 \pm 11.8	26–64	3.1 \pm 1.9	0.4–6.9	49.6 \pm 10.4	31–64	5	5	10
P-Chip	38	50.6 \pm 10.0	26–67	3.4 \pm 1.7	0.35–8.1	51.3 \pm 9.4	28–68	15	11	12

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retention. Tags were considered lost if the scanner could not detect a tag for the remainder of the trial. Tag readability, i.e., the ability to obtain the identification code of an implanted p-Chip tag, was assessed over time by novice scanners monthly. A novice scanner, i.e., someone who never scanned or had experience with p-Chips before participating in this study, scanned a subset of at least 20% of the salamanders each month to assess any tag readability differences between the experienced and novice scanners. The subset was selected by randomly selecting a tank tagged group and requiring the novice scanner to scan all individuals in that group. A new novice scanner was used each month to examine readability across many individuals and avoid bias due to any experience gained over the duration of the study. Readability was quantified as the percentage of salamanders successfully scanned by the novice scanner. Salamanders were not anesthetized during scanning events to reduce unnecessary stress. There were not enough participants to have a novice scanner for each species each month; so, the order in which salamander species were scanned varied across novice scanners to reduce bias due to any experience gained during the scanning process. SVLs were recorded at the conclusion of the study to determine if growth was affected by tagging, although final weights were not recorded to reduce unnecessary salamander stress.

Analyses. Kaplan-Meier time-at-event curves (Goel et al. 2010) were built to examine survival and retention over time. These curves estimated the probability of an event (survival or retention) occurring at each time interval. Days post tagging and weeks post tagging were used as the time increments for survival and retention, respectively. This approach could reveal any differences across time that may be missed with other methods. For example, high mortality immediately following tagging might be an indication of harm from tagging even if survival rates are somewhat similar across groups. The two null hypotheses tested using log-rank tests were that survival curves did not differ among treatments or by sex for each species and that retention curves did not differ among species. Only salamanders that could be sexed were included in tests comparing survival between sexes (Table 1). Data for juvenile and adult Texas Blind Salamanders were pooled due to the similarity in results. Differences were considered significant at $\alpha \leq 0.05$. Kaplan-Meier curves and log-rank tests were performed in the “survival” package (Therneau 2020) in the program R version 4.2.2 (R Core Team 2022).

Tag readability over time and the effects of tagging on growth were examined. Tag readability was assessed using Pearson’s pairwise correlation coefficient to determine the correlation between the percentage of scanned tags to the time since tagging in months. A correlation was considered to be strong at $|r| \geq 0.50$. One-way ANOVAs were performed to confirm that there were

no differences in initial SVLs among treatments for each species. To determine the effects of tagging on growth, two-sample t-tests were conducted to compare the growth of tagged salamanders to control salamanders for each species. Growth was calculated by subtracting the initial SVL from the final SVL of each salamander, and differences were considered significant at $\alpha \leq 0.05$. All assumptions for analyses (normality, homoscedasticity, independence, and no outliers) were met by the data in this study. The base package in the program R version 4.2.2 (R Core Team 2022) was used to calculate the correlation coefficients and conduct the t-tests.

Results

Tagging salamanders with p-Chips had no effect on their survival, and no difference in survival was evident between male and female salamanders (Figs. 2–4). Survival did not differ among treatment groups for Barton Springs Salamander ($\chi^2 = 0.5$, $p = 0.8$), Comal Springs Salamander ($\chi^2 = 2.3$, $p = 0.3$), or Texas Blind Salamander ($\chi^2 = 1.1$, $p = 0.6$). Additionally, survival did not differ between males and females among the treatment groups for Barton Springs Salamander ($\chi^2 = 4.5$, $p = 0.5$), Comal Springs Salamander ($\chi^2 = 5.2$, $p = 0.4$), or Texas Blind Salamander ($\chi^2 = 2.4$, $p = 0.8$). Three Barton Springs Salamander mortalities (two tagged and one control) occurred on day 138 of the study, and an additional mortality in the sham group occurred on day 177 (Fig. 2). One Comal Springs Salamander mortality occurred in the control group on day 150 (Fig. 3), and one adult Texas Blind Salamander mortality occurred in the tagged group on day 191 (Fig. 4). Tag retention was relatively high and did not differ among species ($\chi^2 = 1.1$, $p = 0.06$; Table 2). One Barton Springs Salamander tag was lost in week 1 of the study, and one tag in a Comal Springs Salamander was either lost or shifted to the point that it could not be read in week 6 of the study (Fig. 5).

Tag readability for Barton Springs Salamanders varied across novice scanners, but all Comal Springs Salamander and Texas Blind Salamander tags were readable by every novice (Table 3). There was not a strong correlation between readability and time since tagging for Barton Springs Salamanders over the eight months of this study ($r = 0.31$). Only the experienced scanner was able to read one of the Barton Springs Salamander tags. This salamander was randomly selected for reading by novice scanners in months 1–4 and 6. Novice scanners in months 3 and 4 were unable to read other salamander p-Chip tags, but those tags were successfully read by novice scanners thereafter (Table 3). The novice scanners in months 3 and 4 both attempted to read the Barton Springs Salamanders first and then read the other species’ tags afterward. All novice tag scanners were able to accurately read all Comal Springs Salamander and Texas Blind Salamander tags throughout the study.

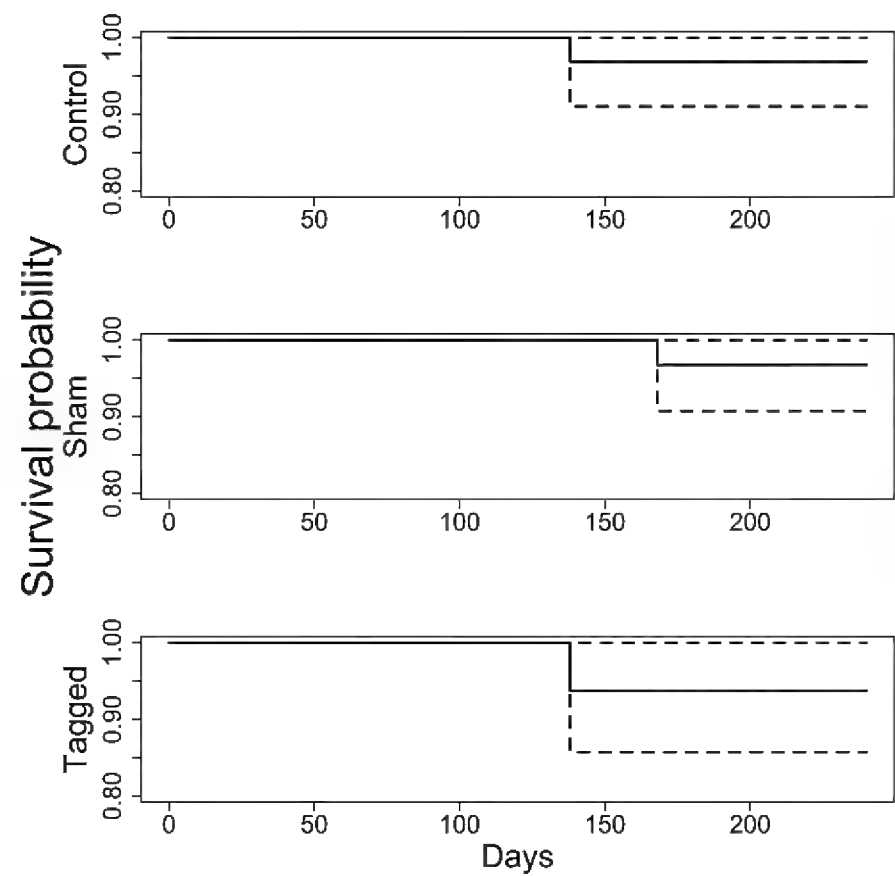


Fig. 2. Kaplan-Meier survival curves for Barton Springs Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

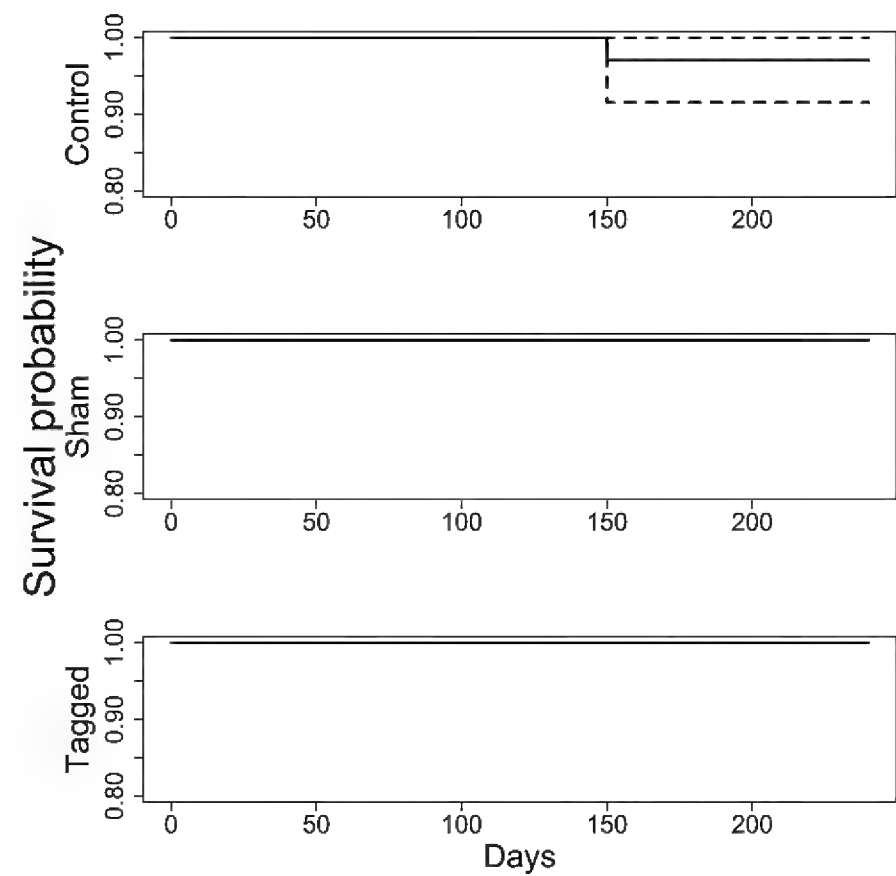


Fig. 3. Kaplan-Meier survival curves for Comal Springs Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

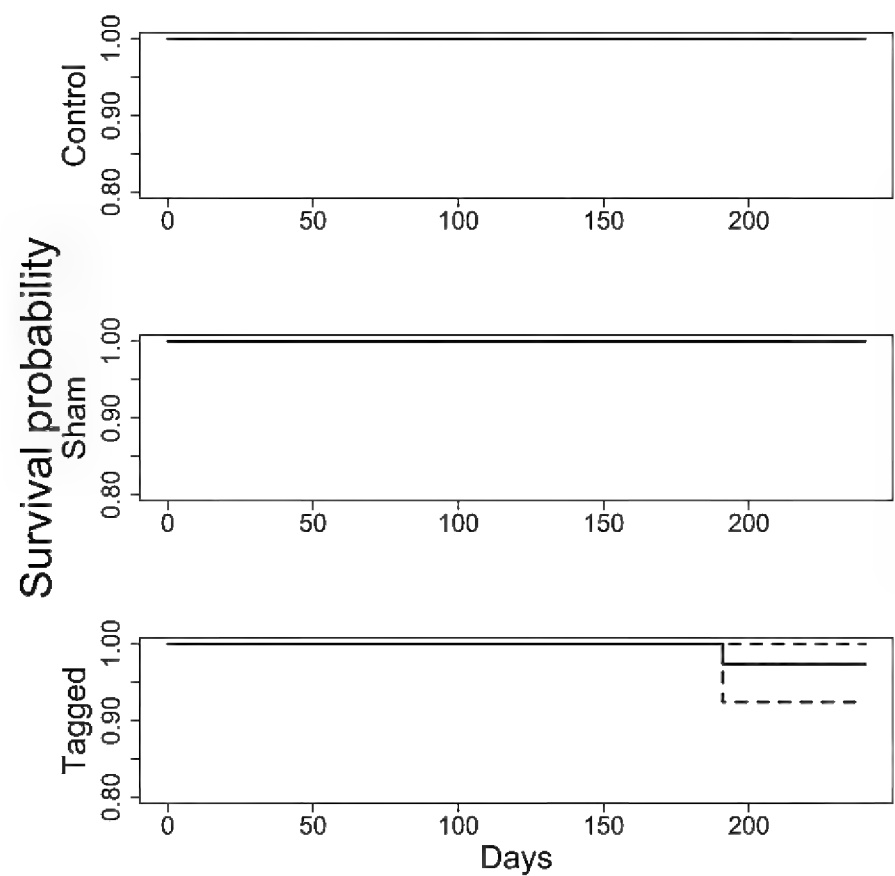


Fig. 4. Kaplan-Meier survival curves for Texas Blind Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

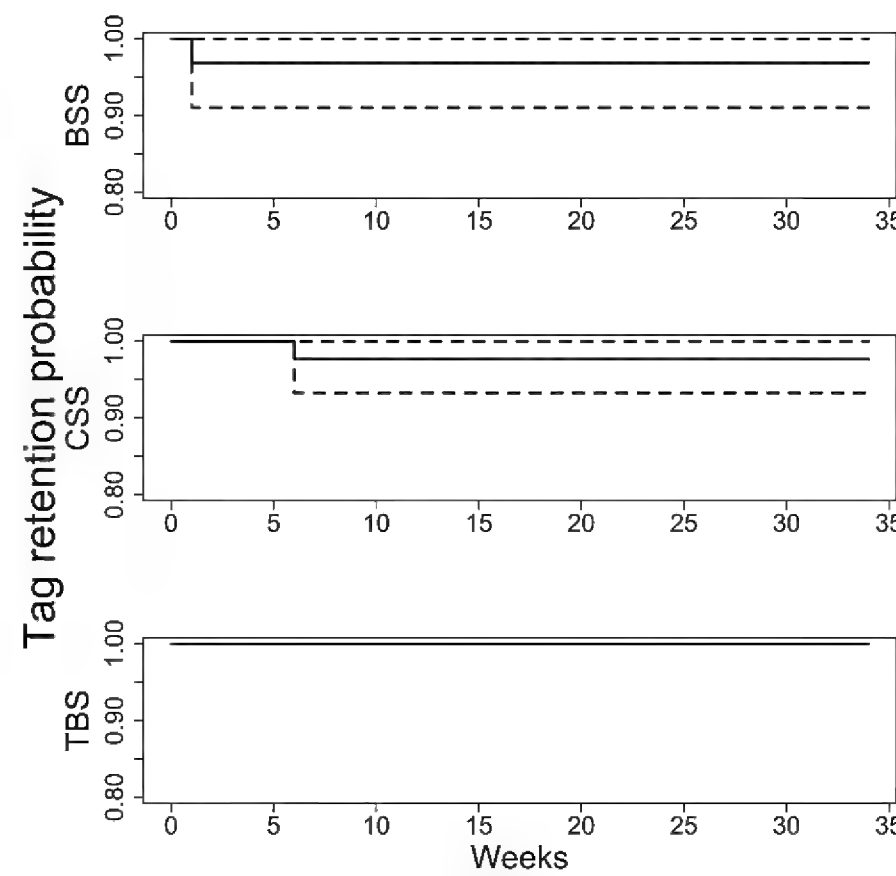


Fig. 5. Kaplan-Meier p-Chip retention curves for Barton Springs Salamanders (BSS), Comal Springs Salamanders (CSS), and Texas Blind Salamanders (TBS). The probability of p-Chip retention is shown with 95% confidence intervals (dashed lines) over time in weeks since tagging.

Growth was not affected by tagging in any of the three species. Initial lengths and weights were closely correlated (Pearson’s product moment coefficient $|r| = 0.93$), and so the lengths were used for growth analyses. The initial lengths did not differ among treatments for Barton Springs ($F_2, 92 = 1.39, p = 0.25$), Comal Springs ($F_2, 108 = 0.39, p = 0.68$), or Texas Blind ($F_2, 75 = 0.47, p = 0.63$) salamanders. Although the final mean SVL was smaller than the initial mean SVL for some groups

(Table 1), growth did not differ between the tagged and control groups for Barton Springs Salamander ($p = 0.84$), Comal Springs Salamander ($p = 0.53$), or Texas Blind Salamander ($p = 0.64$). Visible growth was noted in the juvenile Texas Blind Salamanders, but not in any other groups. Although not formally examined, we noted that the control and tagged groups of Comal Springs and Texas Blind Salamanders produced multiple clutches of viable eggs over the duration of the study.

Discussion

Improving tagging methods for small-bodied aquatic organisms is important for conservation and management, and progress is underway with new technological developments. P-Chips resulted in high survival (97–100%) and tag retention (97–100%) without inhibiting growth in aquatic salamanders, indicating the potential for using p-Chip tags in lab and field studies involving aquatic salamander species. Additionally, p-Chips provided an improved readability rate, reduced human error, and allowed for a greater number of individual identification codes than the VIE tags commonly used for these species. To our knowledge, this is the first study examining p-Chips in aquatic salamander species.

We found p-Chips to be appropriate and versatile tags for aquatic salamanders. P-Chips provided high survival and retention rates in Barton Springs, Comal Springs, and Texas Blind Salamanders. Our results are similar to those of studies examining p-Chips in other aquatic organisms (Chen et al. 2013; Faggion et al. 2020; Moore and Brewer 2021). Although photo identification has been used in Barton Springs Salamanders (Bendick et al. 2021), we are unaware of any other published studies examining the efficacy of photo identification in any of the species in our study. Photo identification is labor intensive as it requires time to take and process the photos. Tagging and scanning p-Chips requires only seconds for an experienced tagger. P-Chips would be preferred to photo identification when time is a concern and an experienced tagger is available. P-Chips were more versatile than the previously used VIE tags. Although survival and tag retention were similar in previous VIE tagging research (Phillips and Fries 2009; Moon et al. 2022), p-Chips provided individual identification with a single tag. To achieve the same resolution of individual information provided by a single p-Chip tag, especially in large sample sizes, VIE tag codes would require the injection of multiple tags per individual. Increasing the number of wounds might increase animal stress, the possibility of infection, and mortality over time. P-Chips also enabled individual identification in small (e.g., ≤ 35 mm SVL) salamanders that might not be able to survive the injection of several VIE tags. There were fewer opportunities for human error when using p-Chips because tag codes were recorded directly from the laser reader into a CSV file instead of being manually observed, interpreted, and written or typed. Additionally, novice scanners were more successful at reading p-Chips (100%) compared to novice scanners reading VIE tags in Comal Springs and Texas Blind salamanders (Moon et al. 2022). It is not currently known whether p-Chips would perform as well when applied to salamanders in a wild setting where habitats are more variable and predators might be present.

Although tag readability was optimal for Comal Springs and Texas Blind Salamanders, novice scanners made occasional errors when reading p-Chips in Barton Springs Salamanders. Unlike with VIE tags in *Eurycea* spp. (Moon et al. 2022), tag readability in Barton Springs Salamanders was not related to time since tagging. Instead, the difficulty reading tags seemed to be related to individual salamander tag placement and variations in scanner ability, and was unique to the Barton Springs Salamanders in this study. For example, one individual salamander was unreadable by all novice scanners who attempted to scan it. The experienced scanner noted that the tag in this salamander was at an angle, and the tag had to be read by pointing the laser upward from the underside of the salamander. The novice scanners did not have the experience to identify and troubleshoot this issue and were unable to read that tag. This instance indicates that the experience of the tagger might be important for overall readability, since a less experienced tagger might not be able to tag as many individuals with consistent placement regarding depth, angle, and location. Another possibility is that readability may have been increased for the tagger, as hypothesized with VIE tags (Moon et al. 2022). The wide range of readability scores (50–100%) indicated that individual scanner variation might affect novice readability in Barton Springs Salamanders. Possible reasons for this variation include variation in eyesight, patience, interest, and similar experiences. For example, individuals that have read other types of tags in the past might be more able to read p-Chips without direct experience.

It is notable that readability issues were only present for Barton Springs Salamanders, indicating there might be some anatomical or behavioral traits that reduce readability overall. Another possibility is that this issue was partially due to novice scanners having more trouble with the first species they read. However, this issue was not seen for novice scanners that read other species first, and some scanners that began with other species were not able to read all the Barton Springs Salamander tags. On several occasions, the experienced and novice scanners noted that the Barton Springs Salamanders seemed more physically active than the other species, so it was difficult to keep them positioned long enough to find the proper angle for reading. Anesthetizing salamanders during the scanning event might improve the ability of novice scanners to read these tags. However, repeated anesthetization in these species is not well studied and might have negative effects. Additionally, the Barton Springs Salamanders are more pigmented than Texas Blind Salamanders and seem to have thicker skin than Comal Springs Salamanders, a trend that might be related to size (i.e., larger salamanders tended to have thicker skin during tagging). Future work should examine the differences among species and ontogenetic stages that could be contributing to this readability issue, which could indicate those species that are most suitable for using p-Chip tags.

The data indicated no evidence that growth was affected by p-Chip tagging or contributed to the migration of the p-Chips. Like VIE tagging in San Marcos Salamanders (Phillips and Fries 2007), growth was unaffected by p-Chip tagging in Barton Springs, Comal Springs, and Texas Blind Salamanders in this study. However, we only examined growth at the conclusion of the study rather than at various points throughout the study, so we may have missed any variation in growth rates earlier in the study (e.g., Baras et al. 1999; Ruetz et al. 2006). Additionally, we did not examine any other metrics that might have been affected by tagging, such as behavior. Growth often affects the migration of subcutaneous tags in aquatic organisms (Linnane and Mercer 1998; Haddaway et al. 2011). For example, growth was shown to increase VIE breakage and deterioration in *Eurycea* spp. (Moon et al. 2022). However, we found no evidence of p-Chip migration with growth. Juvenile Texas Blind Salamanders had the highest growth rate but were also associated with 100% readability and tag retention. Growth might affect the migration of p-Chips used in individuals smaller than the ones we examined. More research is needed to evaluate the use of p-Chips in smaller (≤ 25 mm SVL) individuals of each of these species.

The results of this study show that P-Chips are suitable tags for small-bodied aquatic salamanders, especially for projects that require individual identification. However, other tags such as VIE tags might be more appropriate in projects where individual identification is not needed or short-term studies with few individuals, especially when costs must be reduced. The duration of effectiveness for subcutaneously injected p-Chips in aquatic organisms remains unknown. Longer-term monitoring is needed to determine the endpoint of p-Chip efficacy in long-lived aquatic organisms. Although no deterioration of p-Chip readability was observed over the eight months of this study, they may become more difficult to read with age. Additionally, more research is needed to determine the size limits of salamanders that can be tagged with p-Chips, particularly in the smaller salamander species in which we only tagged adults. Tagging juveniles could be beneficial for examining recruitment and growth rates, and for tracking individual metrics such as genetic, collection, and rearing information. Research comparing the efficacies of photo and p-Chip identification in these species is needed. Photo identification is an effective method of the identification for some species at a low cost and might be preferable to, or used in conjunction with, p-Chips for some projects. Although we found no effect on growth from tagging with p-Chips, additional studies examining the effects of p-Chip tagging on behaviors such as swimming, hunting, and reproduction are recommended. Because survival and retention are often different in captive and wild settings (e.g., Musselman et al. 2017), studies examining the effects of p-Chips on organisms in the wild are needed to confirm the utility of this tagging method under wild conditions.

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Literature Cited

- Andreone F. 1986. Considerations on marking methods in newts, with particular reference to a variation of the “belly pattern” marking technique. *Bulletin British Herpetological Society* 16: 36–37.
- Baras E, Westerloppe L, Mélard C, Philippart J-C, Bénech V. 1999. Evaluation of implantation procedures for PIT-tagging juvenile Nile Tilapia. *North American Journal of Aquaculture* 61: 246–251.
- Barton Springs/Edwards Aquifer Conservation District. 2018. Final habitat conservation plan for managed groundwater withdrawals from the Barton Springs Segment of the Edwards Aquifer. Barton Springs/Edwards Aquifer Conservation District, Austin, Texas, USA.
- Bendik NF, Morrison TA, Gluesenkamp AG, Sanders MS, O'Donnell LJ. 2013. Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. *PLoS One* 8(3): 59424.
- Bendik NF, Chamberlain DA, Devitt TJ, Donelson SE, Nissen B, Owen JD, Robinson D, Sissel BN, Sparks K. 2021. Subterranean movement inferred by temporary emigration in Barton Springs Salamanders (*Eurycea sosorum*). *PeerJ* 9: 11246.
- Carlson BE, Langkilde T. 2013. A common marking technique affects tadpole behavior and risk of predation. *Ethology* 119(2): 167–177.
- Catalano MJ, Chipps SR, Bouchard MA, Wahl DH. 2001. Evaluation of injectable fluorescent tags for marking centrarchid fishes: retention rate and effects on vulnerability to predation. *North American Journal of Fisheries Management* 21(4): 911–917.
- Chen CH, Durand E, Wang J, Zon LI, Poss KD. 2013. Zebrafish transgenic lines for in vivo bioluminescence imaging of stem cells and regeneration in adult Zebrafish. *Development* 140: 4,988–4,997.
- Cooke SJ, Nguyen VM, Murchie KJ, Thiem JD, Donaldson MR, Hinch SG, Brown RS, Fisk A. 2013. To tag or not to tag: animal welfare, conservation, and stakeholder considerations in fish tracking studies that use electronic tags. *Journal of International Wildlife Law & Policy* 16: 352–374.

- Davis TM, Ovaska K. 2001. Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *Journal of Herpetology* 35: 217–225.
- Devitt TJ, Wright AM, Cannatella DC, Hillis DM. 2019. Species delimitation in endangered groundwater salamanders: implications for aquifer management and biodiversity conservation. *Proceedings of the National Academy of Sciences of the United States of America* 116(7): 2,624–2,633.
- Donnelly MA, Guyer C, Juterbock EJ, Alford RA. 1994. *Techniques for Marking Amphibians*. Smithsonian Institution Press, Washington, DC, USA. 284 p.
- Edwards Aquifer Authority. 2012. Edwards Aquifer recovery implementation program, habitat conservation plan. Edwards Aquifer Authority, San Antonio, Texas, USA.
- Faggion S, Sanchez P, Vandeputte M, Clota F, Vergnet A, Blanc MO, Allal F. 2020. Evaluation of a European Sea Bass (*Dicentrarchus labrax* L.) postlarval tagging method with ultra-small RFID tags. *Aquaculture* 520: 734945.
- Fischer EK, Alvarez H, Lagerstrom KM, McKinney JE, Petrillo R, Ellis G, O'Connell LA. 2020. Neural correlates of winning and losing fights in poison frog tadpoles. *Physiology & Behavior* 223: 112973.
- Fiske P, Amundsen T. 1997. Female bluethroats prefer males with symmetric color bands. *Animal Behavior* 54: 81–87.
- Frommen JG, Hanak S, Schmidl CA, Thünken T. 2015. Visible implant elastomer tagging influences social preferences of Zebrafish (*Danio rerio*). *Behaviour* 152(12–13): 1,765–1,777.
- Gamble L, Ravela S, McGarigal K. 2008. Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the Marbled Salamander, *Ambystoma opacum*. *Journal of Applied Ecology* 45(1): 170–180.
- Gibbons WJ, Andrews KM. 2004. PIT tagging: simple technology at its best. *Bioscience* 54(5): 447–454.
- Gillette JR, Peterson MG. 2001. The benefits of transparency: candling as a simple method for determining sex in Red-backed Salamanders (*Plethodon cinereus*). *Herpetological Review* 32: 233.
- Goel M, Khanna P, Kishore J. 2010. Understanding survival analysis: Kaplan–Meier estimate. *International Journal of Ayurveda Research* 1: 274–278.
- Haddaway NR, Mortimer RJG, Christmas M, Dunn AM. 2011. A review of marking techniques for Crustacea and experimental appraisal of electric cauterization and visible implant elastomer tagging for *Austropotamobius pallipes* and *Pacifastacus leniusculus*. *Freshwater Crayfish* 18(1): 55–67.
- Heemeyer JL, Homyack JA, Haas CA. 2007. Retention and readability of visible implant elastomer marks in eastern Red-backed Salamanders (*Plethodon cinereus*). *Herpetological Review* 38: 425.
- Jepsen N, Thorstad EB, Havn T, Lucas MC. 2015. The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry* 3: 1–23.
- Jungwirth A, Balzarini V, Zöttl M, Salzmann A, Taborsky M, Frommen JG. 2019. Long-term individual marking of small freshwater fish: the utility of visual implant elastomer tags. *Behavioral Ecology and Sociobiology* 73(4): 1–11.
- Linnane A, Mercer JP. 1998. A comparison of methods for tagging juvenile lobsters (*Homarus gammarus* L.) reared for stock enhancement. *Aquaculture* 163: 195–202.
- Measey GJ, Gower DJ, Oommen OV, Wilkinson M. 2001. Permanent marking of a fossorial caecilian, *Gegeneophis ramswamii* (Amphibia: Gymnophiona: Caeciliidae). *Journal of South Asian Natural History* 5: 141–147.
- Moon LM, Butler M, Campbell LG. 2022. Evaluation of tagging methods for unique identification of individuals in three aquatic *Eurycea* salamander species. *Ichthyology & Herpetology* 110: 77–86.
- Moore DM, Brewer SK. 2021. Evaluation of visual implant elastomer, PIT, and p-Chip tagging methods in a small-bodied minnow species. *North American Journal of Fisheries Management* 41: 1,066–1,078.
- Murray DL, Fuller MR. 2000. A critical review of the effects of marking on the biology of vertebrates. Pp. 15–64 In: *Research Techniques in Animal Ecology: Controversies and Consequences*. Editors, Boitani L, Fuller T. Columbia University Press, New York, New York, USA. 464 p.
- Musselman WC, Worthington TA, Mouser J, Williams DM, Brewer SK. 2017. Passive integrated transponder tags: review of studies on warmwater fishes with notes on additional species. *Journal of Fish and Wildlife Management* 8: 353–364.
- Northwest Marine Technologies Inc. 2019. Instructions for visible implant alpha tags. Anacortes, Washington, DC, USA.
- Northwest Marine Technologies Inc. 2021. Instructions for visible implant elastomer (VIE) tags. Anacortes, Washington, DC, USA.
- Osborn MS, Hocking DJ, Conner CA, Peterman WE, Semlitsch RD. 2011. Use of fluorescent visible implant alphanumeric tags to individually mark juvenile ambystomatid salamanders. *Herpetological Review* 42: 43–47.
- Petranka JW. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, DC, USA. 592 p.
- PharmaSeq. 2020. Zebrafish p-Chip implantation protocol. PharmaSeq, Monmouth Junction, New Jersey, USA.
- Phillips CT, Fries JN. 2009. An evaluation of visible implant elastomer for marking the federally listed

- Fountain Darter and the San Marcos Salamander. *North American Journal of Fisheries Management* 29(3): 529–532.
- Phillott AD, Skerratt LF, McDonald KR, Lemckert FL, Hines HB, Clarke JM, Alford RA, Speare R. 2007. Toe-clipping as an acceptable method of identifying individual anurans in mark-recapture studies. *Herpetological Review* 38: 305–308.
- R Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <https://www.R-project.org/>.
- Ricker WE. 1956. Uses of marking animals in ecological studies: the marking of fish. *Ecology* 37(4): 665–670.
- Ruetz CR, Earl BM, Kohler SL. 2006. Evaluating passive integrated transponder tags for marking Mottled Sculpins: effects on growth and mortality. *Transactions of the American Fisheries Society* 135: 1,456–1,461.
- Silvy NJ, Lopez RR, Peterson MJ. 2005. Wildlife marking techniques. *Techniques for Wildlife Investigations and Management* 6: 339–376.
- Sinsch U. 1997. Postmetamorphic dispersal and recruitment of first breeders in a *Bufo calamita* metapopulation. *Oecologia* 112(1): 42–47.
- Therneau T. 2020. A package for survival analysis in R. R package version 3.2-7. Available: <https://CRAN.R-project.org/package=survival>. [Accessed: April 2023].
- Waddle JH, Rice KG, Mazzotti FJ, Percival HF. 2008. Modeling the effect of toe-clipping on treefrog survival: beyond the return rate. *Journal of Herpetology* 42: 467–473.
- Weick SE, Knutson MG, Knights BC, Pember BC. 2005. A comparison of internal and external radio transmitters with Northern Leopard Frogs (*Rana pipiens*). *Herpetological Review* 36: 415–421.
- Wright K. 2001. Restraint techniques and euthanasia. Pp. 111–122 In: *Amphibian Medicine and Captive Husbandry*. Editor, Whitaker KWB. Krieger Publishing Company, Malabar, Florida, USA. 570 p.



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The herpetofauna of Copia Nature Reserve, Vietnam

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Abstract.—We report the results of a herpetofauna inventory of Copia Nature Reserve, Vietnam conducted between November 2012 and June 2020, comprising 48 species of amphibians and 67 species of reptiles, with 105 of the species recorded directly in this study. Four species, *Gracixalus jinxiuensis*, *Dopasia ludovici*, *Hebius boulengeri*, and *Ovophis makazayazaya*, represent new records for Son La Province, and 25 species are recorded for the first time from the Copia Nature Reserve, comprising 11 species of frogs (*Xenophrys maosonensis*, *Microhyla mukhlesuri*, *Limnonectes bannaensis*, *Odorrana chapaensis*, *O. chloronota*, *O. jindongensis*, *O. graminea*, *O. nasica*, *Kurixalus bisacculus*, *Raorchestes parvulus*, and *Rhacophorus kio*), two species of lizards (*Calotes emma* and *Eutropis multifasciatus*), and 12 species of snakes (*Calamaria pavimentata*, *Dendrelaphis ngansonensis*, *Elaphe moellendorffi*, *E. taeniura*, *Euprepiophis mandarinus*, *Lycodon fasciatus*, *L. futsingensis*, *Oligodon fasciolatus*, *Sibynophis collaris*, *Psammodynastes pulverulentus*, *Trimerodytes percarinatus*, and *Pareas carinatus*). The herpetofauna of Copia Nature Reserve has a high level of conservation concern, including eight species listed in the Governmental Decree No. 84/2021/ND-CP, 17 species listed in the Vietnam Red Data Book (2007), 13 species listed in the IUCN Red List (2023), and eight species listed in the CITES Appendices (2023). In addition, we provide data on distribution, natural history, and figures for all the amphibian and reptile species in Copia Nature Reserve, Vietnam.

Keywords. Amphibians, biodiversity, distribution, natural history, new records, reptiles

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Introduction

The Copia Nature Reserve was established in November 2002 by the the People’s Committee of Son La Province with an area of 11,996 ha. The nature reserve is situated in Thuan Chau District and is one of the five protected areas in Son La Province, northern Vietnam (The People’s Committee of Son La Province 2019). The topography of the nature reserve is characterized by steep and mountainous terrain with elevations from 500 to 1,800 m asl. In terms of the herpetofaunal diversity, Le et al. (2009) provided the first list of 12 amphibian and 35 reptile species from

Copia Nature Reserve (NR). Further new records of reptiles and amphibians from this nature reserve were documented by Pham et al. (2012, 2013, 2014b,c, 2015, 2017, 2018, 2019, 2020, 2022), and by Pham and Nguyen (2018). Most recently, two new species of amphibians and a new snake were described from Copia NR, namely *Tylototriton anguliceps* Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes, and Nguyen, 2015 (Le et al., 2015a), *Gracixalus truongi* Tran, Pham, Le, Nguyen, Ziegler, and Pham, 2023 (Tran et al. 2023), and *Achalinus timi* Ziegler, Nguyen, Pham, Nguyen, Pham, Van Schingen, Nguyen, and Le, 2019 (Ziegler et al. 2019). In addition, six new country

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records of amphibians and reptiles have been reported from Copia NR, namely *Boulenophrys daweimontis*, *Leptobrachella eos*, *L. minima*, *Leptobrachium masatakasatoi*, *Amolops vitreus*, and *Parafimbrios lao* (Pham et al. 2014a, 2016; Le et al. 2015b; Nguyen et al. 2015). As a result of our ongoing research over the past ten years, we herein provide an updated list, with new data on the distribution and natural history of amphibians and reptiles from Copia NR.

Materials and Methods

Sixteen field surveys were conducted at six sites in Copia NR, Son La Province, Vietnam, including a total of 95 days. Survey sites were set up in the forests near Huoi Pu and Hua Ty A villages, Chieng Bom Commune; near Nong Vai, Pha Khuo ng, and Co Ma villages, Co Ma Commune; and near Long He Village, Long He Commune by A.V. Pham, T.Q. Nguyen, T.T. Nguyen, N.B. Sung, H.V. Tu, T.V. Nguyen (Fig. 1 and Table 1).

The typical habitats at the study sites were undisturbed evergreen forest, secondary forest, and agricultural areas (Fig. 2). The geographical coordinates (WGS84) of all observations were recorded using a Garmin GPSMAP 62s. Specimens were collected by hand between 0800–2300 h. After they were photographed in life, specimens were identified to the species level, measured, sexed, and released at the collection site. For voucher specimens, a few individuals were anesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmon 2002), fixed in 80% ethanol, and then transferred to 70% ethanol for permanent storage.

Some road-killed specimens were also collected for morphological examination. These specimens were subsequently deposited in the collection of the University of Science, Vietnam National University (VNU), Hanoi, Vietnam.

For taxonomic identification, we referred to the descriptions in Bain et al. (2003), Boulenger (1893), Bourret (1942), Inger et al. (1999), Fei et al. (2012), Hecht et al. (2013), Smith (1935, 1943), and Taylor (1962). For species names, we followed Frost (2023) for amphibians and Uetz et al. (2023) for reptiles.

Conservation status of amphibian and reptile species followed the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023), the Red List of the International Union for Conservation of Nature (IUCN 2023), the Vietnam Red Data Book (Dang et al. 2007), and the Governmental Decree No 84/2021/ND-CP, issued on 22 September 2021 by the Government of Vietnam, on the management of endangered species of wild flora and fauna.

Results

A total of 115 species belonging to 78 genera and 27 families were recorded from Copia NR, comprising 48 species of amphibians (28 genera, seven families) and 67 species of reptiles (50 genera, 20 families) (Table 2). Remarkably, one species of frog, one species of lizard and two species of snakes are reported for the first time from Son La Province; and 25 additional species are documented for the first time from Copia NR, comprising 11 species of anurans, two species of lizards, and 12 species of snakes.

Table 1. Information on the survey sites in Copia Nature Reserve, Vietnam.

No	Site	Survey dates	Latitude	Longitude	Elevation (m)
1	Forest near Huoi Pu Village, Chieng Bom Commune, Thuan Chau District	24 to 30 April 2013; 17 to 24 June 2016	21°23.110'N	103°38'522"E	860
2	Forest near Hua Ty A Village, Chieng Bom Commune, Thuan Chau District	20 to 25 November 2012; 20 to 25 March 2013; 2 to 13 September 2016	21°21.210'N	103°35.566'E	960
3	Forest near Nong Vai Village, Co Ma Commune, Thuan Chau District	11 to 19 June 2013; 16 to 22 October 2016	21°18.589'N	103°33.250'E	1,450
4	Forest near Pha Khuong Village, Co Ma Commune, Thuan Chau District	4 to 15 July 2013	21°21.426'N	103°31.230'E	1,260
5	Forest near Co Ma Village, Co Ma Commune, Thuan Chau District	19 to 24 May 2015	21°21.469'N	103°30.380'E	1,090
6	Forest near Long He Village, Long He Commune, Thuan Chau District	22 to 31 August 2014; 18 to 29 April 2020	21°24.130'N	103°29.238'E	1,010

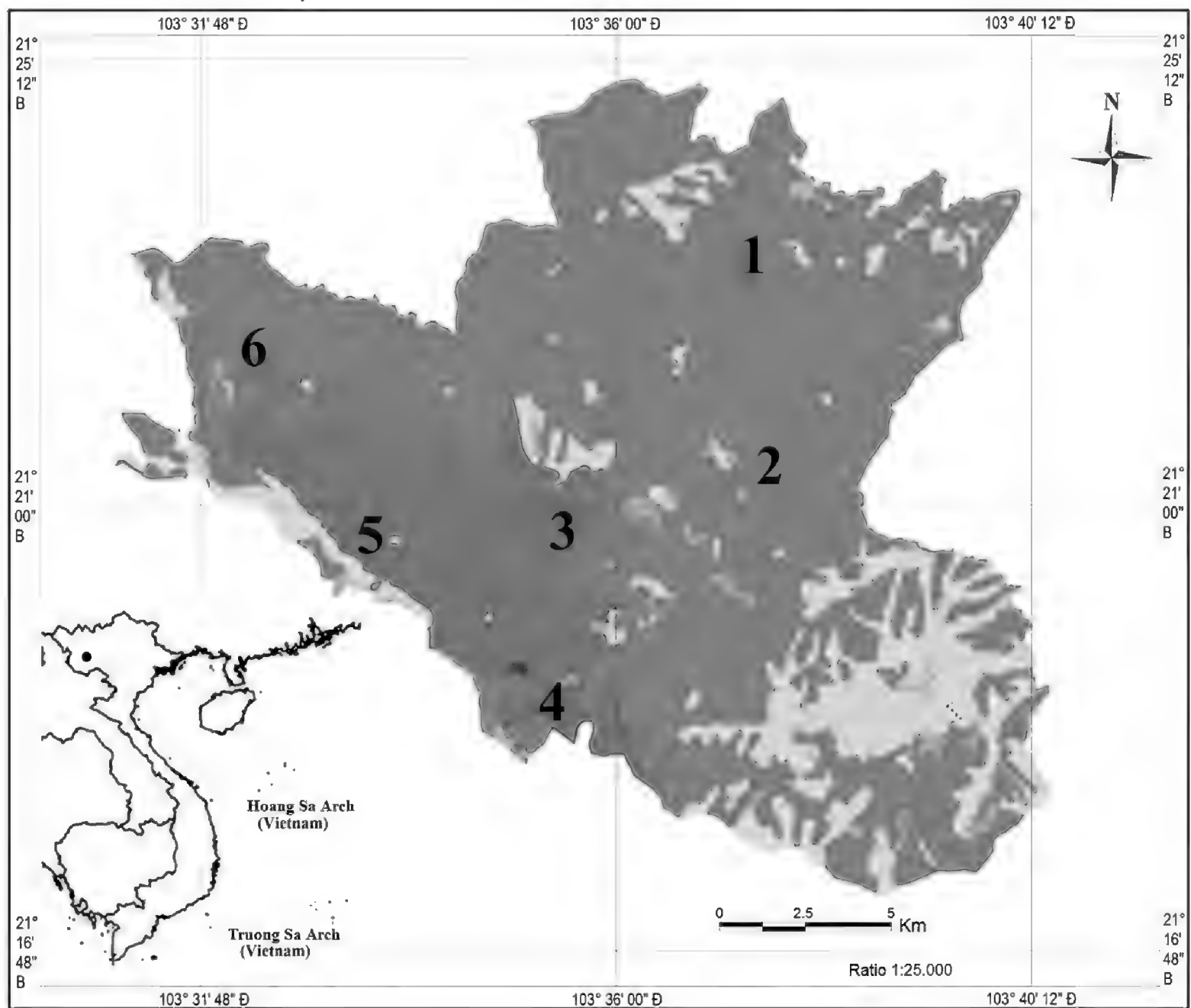


Fig. 1. Survey sites in Copia Nature Reserve, Son La Province, Vietnam. 1. Huoi Pu Village, Chieng Bom Commune; 2. Hua Ty A Village, Chieng Bom Commune; 3. Nong Vai Village, Co Ma Commune; 4. Pha Khuong Village, Co Ma Commune; 5. Co Ma Village, Co Ma Commune; and 6. Long He Village, Long He Commune.

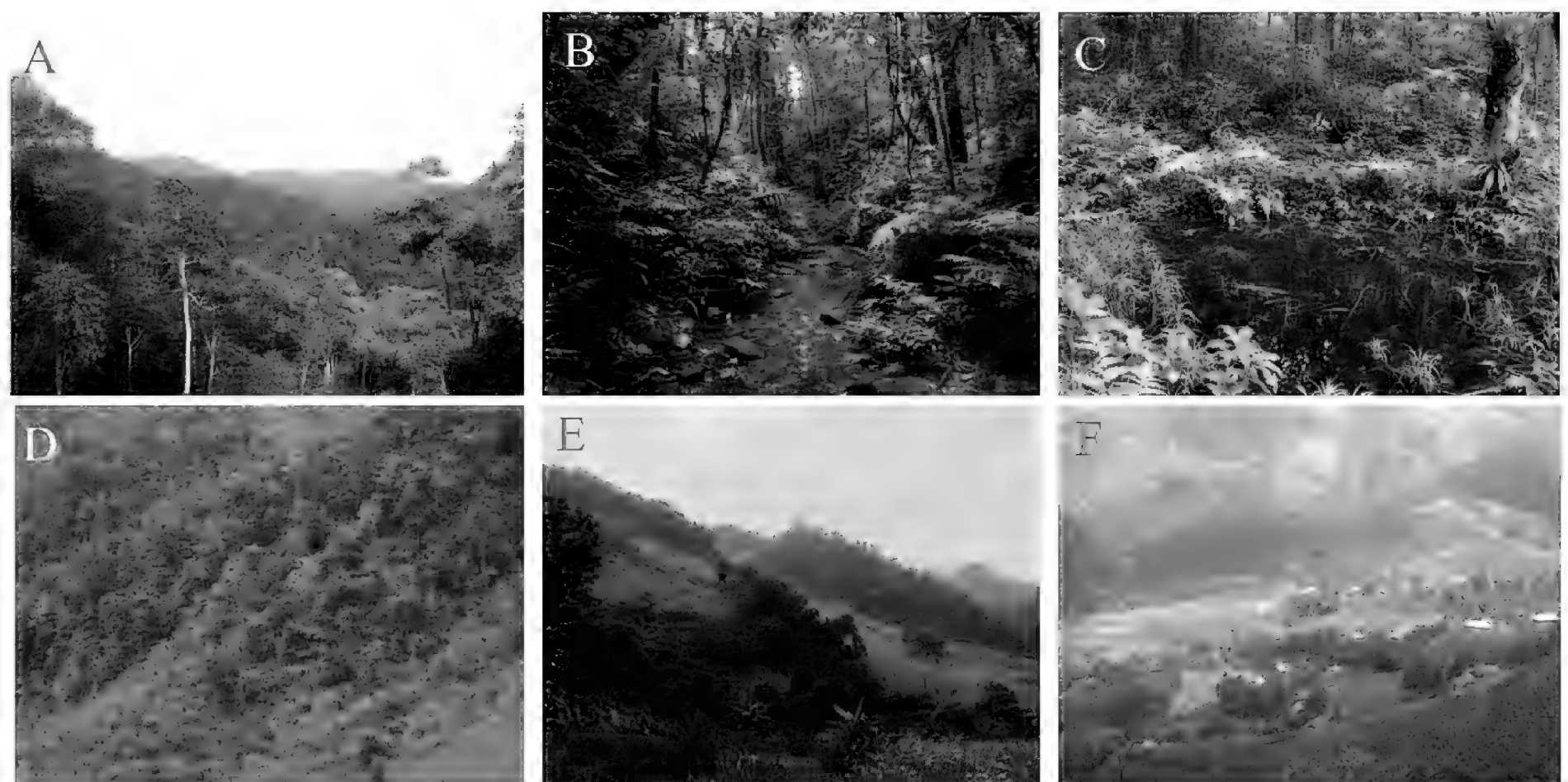


Fig. 2. Habitat types in Copia Nature Reserve, Vietnam. (A, B, C) Evergreen forest, (D) Disturbed secondary forest, and (E, F) Agricultural areas.

Table 2. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023): I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
	ANURA								
	Bufonidae Gray, 1825								
1	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)	1–6	2, 3					Photos	1
	Megophryidae Bonaparte, 1850								
2	<i>Boulenophrys daweimontis</i> (Rao and Yang, 1997)	3	1					Specimens (1)	9
3	<i>Boulenophrys palpebralespinosa</i> (Bourret, 1937)	1, 2	1	CR				Specimens (3) Photos	6
4	<i>Boulenophrys</i> cf. <i>parva</i> (Boulenger, 1893)	1	1					Specimens (2)	15
5	<i>Leptobrachella eos</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler, and Dubois, 2011)	3, 5	1					Specimens (2) Photos	4
6	<i>Leptobrachella minima</i> (Taylor, 1962)	3	1					Specimens (2)	11
7	<i>Leptobrachella ventripunctata</i> (Fei, Ye, and Li, 1990)	1–5	1					Specimens (2) Photos	6
8	<i>Leptobrachium masatakasatoi</i> Matsui, 2013	3, 4	1					Specimens (3)	11
9	<i>Ophryophryne pachyproctus</i> Kou, 1985	1, 3	1					Specimens (2) Photos	6
10	<i>Xenophrys maosonensis</i> (Bourret, 1937)*	1–6	1					Specimens (2) Photos	
	Microhylidae Günther, 1858 (1843)								

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
11	<i>Kaloula pulchra</i> Gray, 1831	1	3					Photos	1
12	<i>Microhyla butleri</i> Boulenger, 1900	1, 2, 4, 6	3					Specimens (2) Photos	1
13	<i>Microhyla heymonsi</i> Vogt, 1911	1–6	2, 3					Specimens (2) Photos	1
14	<i>Microhyla mukhlesuri</i> Hasan, Islam, Kuramoto, Kurabayashi, and Sumida, 2014*	1–6	3					Specimens (2) Photos	
15	<i>Microhyla pulchra</i> (Hallowell, 1861)	1, 2, 4, 5, 6	3					Specimens (3) Photos	1
16	<i>Micryletta menglienica</i> (Yang and Su, 1980)	3	2					Specimens (2)	
	Dicroglossidae Anderson, 1871								
17	<i>Fejervarya limnocharis</i> (Gravenhorst, 1829)	1–6	2, 3					Photos	1
18	<i>Hoplobatrachus chinensis</i> (Osbeck, 1765)	1, 2, 6	3					Photos	1
19	<i>Limnonectes bannaensis</i> Ye, Fei, Xie, and Jiang, 2007*	1–5	1, 2					Specimens (2) Photos	
20	<i>Nanorana aenea</i> (Smith, 1922)	1, 3, 5	1					Specimens (2) Photos	2
21	<i>Quasipaa verrucospinosa</i> (Bourret, 1937)	1–5	1					Photos	1
	Ranidae Batsch, 1796								
22	<i>Amolops</i> cf. <i>compotrix</i> (Bain, Stuart, and Orlov, 2006)	3	1					Specimens (2)	

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a), 5 = Pham et al. (2014b), 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
23	<i>Amolops vitreus</i> (Bain, Stuart, and Orlov, 2006)	3	1		VU			Specimens (2) Photos	9
24	<i>Hylarana macrodactyla</i> Gunther, 1858								1
25	<i>Hylarana taipehensis</i> (Van Denburgh, 1909)								1
26	<i>Odorrana chapaensis</i> (Bourret, 1937)*	5	1					Specimens (2)	
27	<i>Odorrana chloronota</i> (Günther, 1876)*	3, 5	1, 2					Specimens (2) Photos	
28	<i>Odorrana graminea</i> Boulenger, 1900*	3	1		VU			Specimen (1) Photos	
29	<i>Odorrana jingdongensis</i> Fei, Ye, and Li, 2001*	1, 3, 5	1		VU			Specimens (2) Photos	
30	<i>Odorrana nasica</i> (Boulenger, 1903)*	1, 2, 3, 5	1					Specimens (2) Photos	
31	<i>Rana johnsi</i> Smith, 1921								1
32	<i>Sylvirana guentheri</i> (Boulenger, 1882)	1, 2, 4, 5, 6	3					Photos	1
33	<i>Sylvirana nigrovittata</i> (Blyth, 1856)	1–6	1, 2					Specimens (2) Photos	1
	Rhacophoridae Hoffman, 1932 (1858)								
34	<i>Chirixalus doriae</i> Boulenger, 1893	2	2					Specimen (1) Photos	17

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, NT = Vulnerable, VU = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

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35	<i>Gracixalus jinxiuensis</i> (Hu, 1978)**	3	1					Specimens (2)	
36	<i>Gracixalus quang</i> Rowley, Dau, Nguyen, Cao, and Nguyen, 2011	3–5	1					Specimens (3)	2
37	<i>Gracixalus truongi</i> Tran, Pham, Le, Nguyen, Ziegler, and Pham, 2023	6	1					Specimens (2)	18
38	<i>Kurixalus bisacculus</i> (Taylor, 1962)*	1–6	1, 2					Specimens (2) Photos	
39	<i>Polypedates megacephalus</i> Hallowell, 1861*	1–6	1–3					Specimens (2) Photos	
40	<i>Raorchestes parvulus</i> (Boulenger, 1893)*	1, 3, 4, 5	1					Specimens (2) Photos	
41	<i>Rhacophorus kio</i> Ohler, and Delorme, 2006*	1–6	1	EN				Photos	
42	<i>Rhacophorus rhodopus</i> Liu and Hu, 1960	3, 4	1					Specimen (1) Photos	12
43	<i>Theloderma albopunctatum</i> (Liu and Hu, 1962)	1, 3, 5, 6	1					Specimen (1) Photos	13
44	<i>Theloderma bicolor</i> (Bourret, 1937)	3	1					Specimen (1)	13
45	<i>Theloderma corticale</i> (Boulenger, 1903)	3	1	EN				Specimen (1)	13
46	<i>Theloderma gordonii</i> Taylor, 1962	4, 6	1					Specimens (2)	13
47	<i>Zhangixalus dorsovireidis</i> (Bourret, 1937)	3	1		VU			Specimens (2)	12
48	<i>Zhangixalus feae</i> (Boulenger, 1893)	3–5	1	EN				Photos	12

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

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	CAUDATA								
	Salamandridae Goldfuss, 1820								
49	<i>Tylotriton anguliceps</i> Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes, and Nguyen, 2015	3	1			II	IIB	Photos	8
	SQUAMATA								
	Agamidae								
50	<i>Acanthosaura lepidogaster</i> (Cuvier, 1829)	1–6	1, 2					Photos	1
51	<i>Calotes emma</i> Gray, 1845*	1, 2	2, 3					Specimen (1)	
52	<i>Calotes versicolor</i> (Daudin, 1802)	1–6	3					Photos	1
53	<i>Draco maculatus</i> (Gray, 1845)	1, 3, 5	1, 2					Specimen (1)	1
54	<i>Pseudocalotes brevipes</i> (Werner, 1904)	1, 3	1, 2					Specimen (1) Photos	14
	Gekkonidae								
55	<i>Gekko reevesii</i> (Gray, 1831)	1, 3, 5, 6	2					Photos	1
56	<i>Hemidactylus frenatus</i> Duméril and Bibron, 1836	1–6	3					Photos	1
57	<i>Hemidactylus garnotii</i> Duméril and Bibron, 1836	1	2					Specimens (2) Photos	14
	Scincidae								
58	<i>Eutropis longicaudatus</i> (Hallowell, 1857)	1, 2, 3, 6	3					Photos	1

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, NT = Vulnerable, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a), 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

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59	<i>Eutropis multifasciatus</i> (Kuhl, 1820)*	4, 5	3					Photos	
60	<i>Plestiodon</i> cf. <i>tamdaoensis</i> (Bourret, 1937)	1	2					Specimen (1)	
61	<i>Scincella devorator</i> Darevsky, Orlov, and Ho, 2004	3	1					Specimen (1)	7
62	<i>Sphenomorphus indicus</i> (Gray, 1853)	1, 3, 4, 6	1, 2					Specimen (1) Photos	1
63	<i>Tropidophorus baviensis</i> Bourret, 1939	4, 6	2					Specimens (2)	1
	Anguidae								
64	<i>Dopasia harti</i> (Boulenger, 1899)	3	1					Specimen (1)	1
65	<i>Dopasia ludovici</i> (Mocquard, 1905)**	1	1					Specimens (2)	
	Varanidae								
66	<i>Varanus salvator</i> (Laurenti, 1768)			EN		II	IIB		1
	Typhlopidae								
67	<i>Indotyphlops braminus</i> (Daudin, 1803)	3	2					Specimens (2)	1
	Pythonidae								
68	<i>Python bivittatus</i> Kuhl, 1820			CR	NT	II	IIB		1
	Xenopeltidae								
69	<i>Xenopeltis unicolor</i> Reinwardt, 1827	1, 6	3					Specimens (2)	1
	Colubridae								

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a), 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

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70	<i>Ahaetulla prasina</i> (Boie, 1827)	1, 3, 5	2					Photos	1
71	<i>Boiga multomaculata</i> (Boie, 1827)	4	2					Photos	1
72	<i>Calamaria pavementata</i> Duméril, Bibron, and Duméril, 1854*	5	2					Specimen (1)	
73	<i>Coelognathus radiatus</i> (Boie, 1827)	1, 6	3	EN				Photos	1
74	<i>Dendrelaphis ngansonensis</i> (Bourret, 1935)*	3	2					Specimen (1)	
75	<i>Dendrelaphis pictus</i> (Gmelin, 1789)	1, 3	2					Specimens (2)	3
76	<i>Elaphe moellendorffi</i> (Boettger, 1886)*	5	2	VU	VU			Photo	
77	<i>Elaphe taeniura</i> (Cope, 1861)*	5	2		VU			Photos	
78	<i>Euprepiophis mandarinus</i> (Cantor, 1842)*	1	1	VU				Photos	1
79	<i>Gonyosoma coeruleum</i> Liu, Hou, Lwin, Wang, and Rao, 2021	3	1					Specimen (1)	
80	<i>Gonyosoma frenatum</i> (Gray, 1853)	3	1					Specimen (1)	5
81	<i>Liopeltis frenata</i> (Günther, 1858)	3	1					Specimens (2)	5
82	<i>Lycodon futsingensis</i> (Pope, 1928)*	3	1					Specimen (1)	
83	<i>Oligodon catenatus</i> (Blyth, 1854)	3	1					Specimens (2)	5
84	<i>Oligodon fasciolatus</i> (Günther, 1864)*	1	3					Specimen (1)	
85	<i>Oreocryptophis porphyraceus</i> (Cantor, 1839)	3	1	VU				Photos	1
86	<i>Ptyas korros</i> (Schlegel, 1837)	1, 4, 6	2, 3	EN	NT			Photos	1

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023); I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a), 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
87	<i>Ptyas mucosa</i> (Linnaeus, 1758)			EN		II	IIB		1
88	<i>Ptyas multicincta</i> (Roux, 1907)	1	1					Photo	5
89	<i>Sibynophis collaris</i> (Gray, 1853)*	3	1					Specimen (1)	
	Elapidae								
90	<i>Bungarus fasciatus</i> (Schneider, 1801)	5	2	EN				Photo	1
91	<i>Bungarus wanghaotingi</i> Pope, 1928	1, 3, 5, 6	2					Photos	1
92	<i>Naja atra</i> Cantor, 1842	4, 6	2	EN	VU	II	IIB	Photos	1
93	<i>Ophiophagus hannah</i> (Cantor, 1836)			CR	VU	II	IB		1
94	<i>Sinomicrurus maclellandi</i> (Reinhardt, 1844)	3	1					Photo	1
	Homalopsidae								
95	<i>Hypsiscopus plumbea</i> (Boie, 1827)	6	3						1
	Pseudaspididae								
96	<i>Psammodynastes pulverulentus</i> (Boie, 1827)*	5	2					Photos	
	Natricidae								
97	<i>Amphiesma stolatum</i> (Linnaeus, 1758)								1
98	<i>Fowlea flavipunctatus</i> (Hallwell, 1861)	1, 6	3					Photos	1
99	<i>Hebius boulengeri</i> (Gressitt, 1937)**	3	1					Specimens (2)	

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023): I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
100	<i>Hebius chapaensis</i> (Bourret, 1934)	3	1					Specimen (1)	3
101	<i>Rhabdophis chrysargos</i> (Schlegel, 1837)								1
102	<i>Rhabdophis subminiatus</i> (Schlegel, 1837)	2, 6	2, 3					Photos	1
103	<i>Trimerodytes percarinatus</i> (Boulenger, 1899)*	1–3	1					Photos	
	Pareidae								
104	<i>Pareas carinatus</i> (Boie, 1828)*	6	3					Specimen (1)	
105	<i>Pareas hamptoni</i> (Boulenger, 1905)	1–5	1					Specimen (1) Photos	3
106	<i>Pareas margaritophorus</i> (Jan, 1866)	3	1					Specimens (2)	1
	Pseudoxenodontidae								
107	<i>Pseudoxenodon macrops</i> (Blyth, 1854)	3	1					Specimens (2)	
	Viperidae								
108	<i>Ovophis makazayazaya</i> (Takahashi, 1922)**	3	1					Specimens (2)	
109	<i>Ovophis monticola</i> (Günther, 1864)	4	1					Specimens (2)	3
110	<i>Trimeresurus albolabris</i> Gray, 1842	1–6	2, 3					Specimen (1) Photos	1
	Xenodermatidae								

Table 2 Continued. List of amphibian and reptile species recorded from Copia NR, Vietnam: ** = new record for Son La Province; * = new record for Copia NR; Site: Numbering sites from 1 to 6 as shown in Table 1; Habitat: 1 = Evergreen forest, 2 = Disturbed secondary forest, and 3 = Agricultural areas; Red Data Book of Vietnam (Dang et al. 2007); IUCN = The IUCN Red List of Threatened Species (IUCN 2023); CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Lower Risk/Near Threatened; CITES = CITES appendices (CITES 2023): I, II = Appendix I and II; Decree No. 84 = The Governmental Decree No. 84/2021/ND-CP, dated on 22 September 2021, by the Government of Vietnam: IIB = Group IIB (limited exploitation and use for commercial purpose); Citations in the Previous record column: 1 = Le et al. (2009), 2 = Pham et al. (2012), 3 = Pham et al. (2013), 4 = Pham et al. (2014a); 5 = Pham et al. (2014b); 6 = Pham et al. (2014c), 7 = Pham et al. (2015), 8 = Le et al. (2015a), 9 = Le et al. (2015b), 10 = Nguyen et al. (2015), 11 = Pham et al. (2016), 12 = Pham et al. (2017), 13 = Pham and Nguyen (2018), 14 = Pham et al. (2018), 15 = Pham et al. (2019), 16 = Ziegler et al. (2019), 17 = Pham et al. (2022), 18 = Nguyen et al. (2023).

No	Name	Sites	Habitats	Vietnam Red Data Book	IUCN	CITES	Decree No. 84	Record evidence (this study)	Previous record
111	<i>Achalinus timi</i> Ziegler, Nguyen, Pham, Van Schingen, Nguyen, and Le, 2019	3	1						16
112	<i>Parafimbrios lao</i> Teynié, David, Lottier, Le, Vidal, and Nguyen, 2015	3	1						10
	TESTUDINES								
	Platysternidae								
113	<i>Platysternon megacephalum</i> Gray, 1831	1, 6	1	EN	CR	I	IB	Photos	1
	Geoemydidae								
114	<i>Mauremys sinensis</i> (Gray, 1834)				CR				1
	Testudinidae								
115	<i>Manouria impressa</i> (Guenther, 1882)	4, 6	1	VU	EN	II	IIB	Photos	1

Amphibia**Anura****Bufonidae**

Duttaphrynus melanostictus (Schneider, 1799) (Fig. 3A): Individuals were observed at night on the ground in croplands, gardens, and road edges near residential areas and disturbed secondary forest.

Megophryidae

Boulenophrys daweimontis (Rao and Yang, 1997) (Fig. 3B): One specimen was found at night on the ground, near a stream in evergreen forest.

Boulenophrys palpebralespinosa (Bourret, 1937) (Fig. 3C): Two specimens were found at night on leaves, at ca. 30–90 cm above the ground near a stream, and many other individuals were observed along streams at night in evergreen forest.

Boulenophrys cf. *parva* (Boulenger, 1893) (Fig. 3D): Two specimens were found at night on the ground, near a stream in evergreen forest. *Boulenophrys parva* seems to be restricted in Myanmar and records of this species in northern Vietnam should be assigned to other named and unnamed species (Manhony et al. 2020).

Leptobrachella eos (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler, and Dubois, 2011) (Fig. 3E): Two specimens were found at night on the ground near streams and many other individuals were observed at night on the ground along streams in evergreen forest.

Leptobrachella minima (Taylor, 1962) (Fig. 3F): Two specimens were found at night on the ground near a stream in evergreen forest.

Leptobrachella ventripunctata (Fei, Ye, and Li, 1990) (Fig. 3G): Two specimens were found on the ground near streams and many other individuals were observed along streams at night in evergreen forest.

Leptobrachium masatakasatoi Matsui, 2013 (Fig. 3H): Three specimens were found at night on the ground near a stream in evergreen forest.

Ophryophryne pachyproctus Kou, 1985 (Fig. 3I): Two specimens and many others were found at night on leaves, at ca. 50–120 cm above the ground near streams, and many other individuals were observed on leaves along streams at night in evergreen forest.

Xenophrys maosonensis (Bourret, 1937) (Fig. 3J): Two specimens were found at night on the ground near a stream, and many other individuals were observed along streams at night in evergreen forest. This is a new record for Copia NR.

Microhylidae

Kaloula pulchra Gray, 1831 (Fig. 3K): Two individuals were observed at night on the ground in a garden near a pond.

Microhyla butleri Boulenger, 1900 (Fig. 3L): Two specimens were found at night on the ground in meadowlands, and other individuals were observed at night in croplands and small puddle edges near the rice fields and forest edges.

Microhyla heymonsi Vogt, 1911 (Fig. 3M): Two specimens were found at night on the ground in croplands, and other individuals were observed at night on the ground in croplands in meadowlands, and along forest trails near forest edges and inside the forest.

Microhyla mukhlesuri Hasan, Islam, Kuramoto, Kurabayashi, and Sumida, 2014 (Fig. 3N): Two specimens were found at night on the ground near small puddle edges near a road, and other individuals were observed at night on the ground near small puddle edges in rice fields, meadowlands, and croplands. This is a new record for Copia NR.

Microhyla pulchra (Hallowell, 1861) (Fig. 3O): Three specimens were found at night on the ground in meadowlands near rice fields, and other individuals were observed at night on the ground around small puddle edges and in meadowlands near rice fields.

Micryletta menglienica (Yang and Su, 1980) (Fig. 3P): Two specimens were found at night on the ground in meadowlands near limestone mountains. This is a new record for Copia NR.

Dicroglossidae

Fejervarya limnocharis (Gravenhorst, 1829) (Fig. 3Q): Many individuals were observed at night on the ground, as well as in meadowlands near rice fields, croplands, and small puddles at road edges.

Hoplobatrachus chinensis (Osbeck, 1765) (Fig. 3R): Five individuals were observed at night on the ground, and at pond edges and rice field edges.

Limnonectes bannaensis Ye, Fei, Xie, and Jiang, 2007 (Fig. 4A): Two specimens were found at night on the ground near a stream, and many other individuals were observed at night on the ground near streams or water edges of streams in evergreen forest. This is a new record for Copia NR.

Nanorana aenea (Smith, 1922) (Fig. 4B): Two specimens were found at night on the ground near a stream, and many other individuals were observed at night on the ground near streams in evergreen forest.

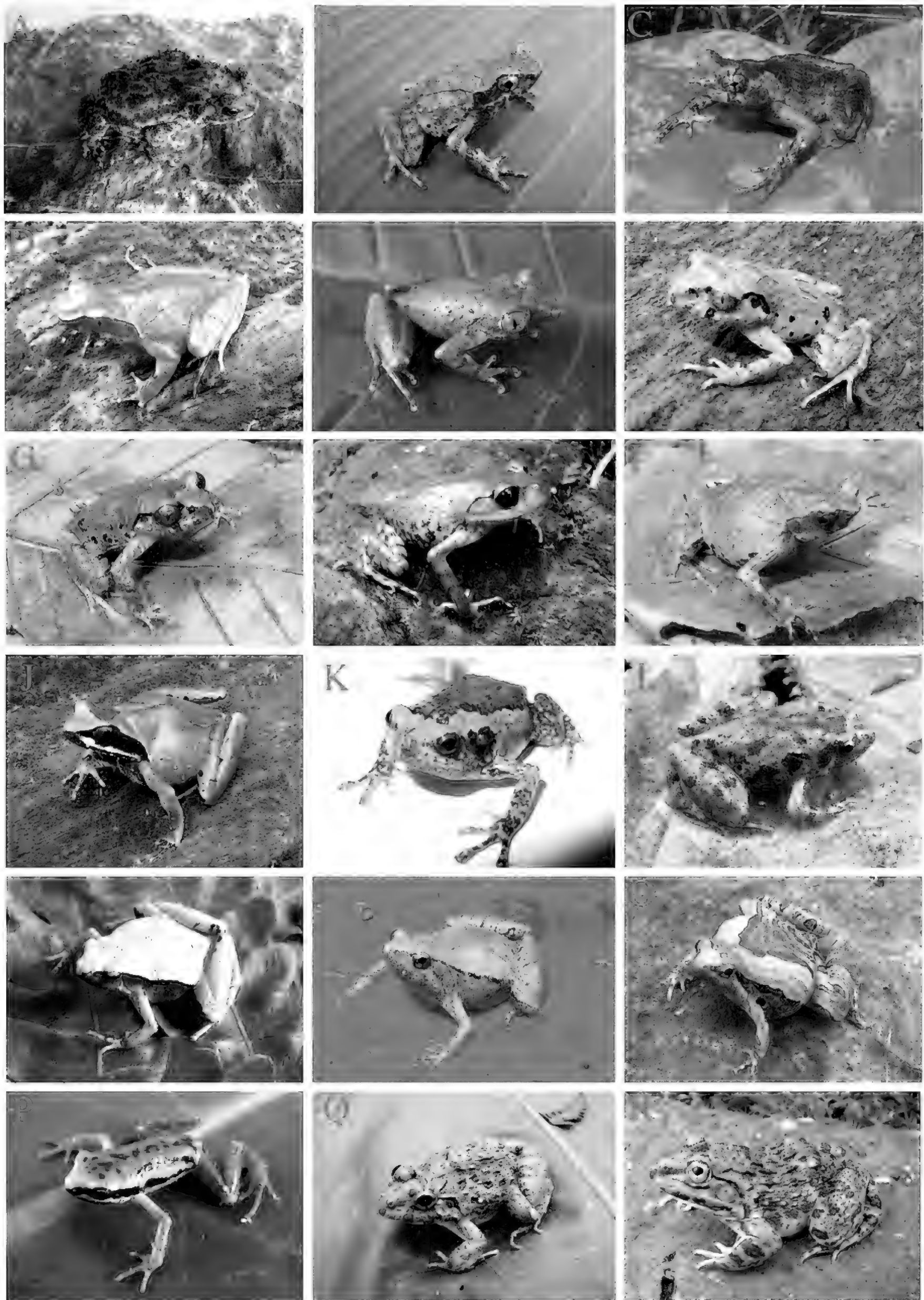


Fig. 3. Amphibian species recorded in Copia Nature Reserve, Vietnam. (A) *Duttaphrynus melanostictus*, (B) *Boulengerophrys dawsoni*, (C–D) *B. Palpebralespinosa*, (E) *Leptobrachella eos*, (F) *L. minima*, (G) *L. ventripunctata*, (H) *Leptobrachium masatakasatoi*, (I) *Ophryophryne pachyproctus*, (J) *Xenophrys maosonensis*, (K) *Kaloula pulchra*, (L) *Microhyla butleri*, (M) *M. heymonsi*, (N) *M. mukhlesuri*, (O) *M. pulchra*, (P) *Micryletta mengliensis*, (Q) *Fejervarya limnocharis*, and (R) *Hoplobatrachus chinensis*.



Fig. 4. Amphibian species recorded in Copia Nature Reserve, Vietnam. (A) *Limnonectes bannaensis*, (B) *Nanorana aenea*, (C) *Quasipaa verrucospinosa*, (D) *Amolops* cf. *compotrix*, (E) *A. vitreus*, (F) *Odorrana chapaensis*, (G) *O. chloronota*, (H) *O. graminea*, (I) *O. jingdongensis*, (J) *O. nasica*, (K) *Sylvirana guentheri*, (L) *S. nigrovittata*, (M) *Chirixalus doriae*, (N) *Gracixalus jinxiuensis*, (O) *G. quangii*, (P) *G. truongi*, (Q) *Kurixalus bisaccus*, and (R) *Polypedates megacephalus*.

Quasipaa verrucospinosa (Bourret, 1937) (Fig. 4C): Individuals were observed at night on rocks in streams or near waterfalls in evergreen forest.

Ranidae

Amolops cf. *compotrix* (Bain, Stuart, and Orlov, 2006) (Fig. 4D): Two specimens were found at night on tree branches near streams. The surrounding habitat was evergreen forest, composed of small hardwoods, liane, and shrub. This is a new record for Copia NR.

Amolops vitreus (Bain, Stuart, and Orlov, 2006) (Fig. 4E): Two specimens were found at night on tree branches near a stream, and six other individuals were observed at night on tree branches near streams in evergreen forest.

Odorrana chapaensis (Bourret, 1937) (Fig. 4F): Two specimens were found at night on a rock near a waterfall in evergreen forest. This is a new record for Copia NR.

Odorrana chloronota (Günther, 1876) (Fig. 4G): Two specimens were found at night on a rock near a stream, and many other individuals were observed at night on tree branches or on rocks near streams in evergreen forest. This is a new record for Copia NR.

Odorrana graminea Boulenger, 1900 (Fig. 4H): One specimen was found at night on a rock near a waterfall in evergreen forest. This is a new record for Copia NR.

Odorrana jingdongensis Fei, Ye, and Li, 2001 (Fig. 4I): Two specimens were found at night on a tree branch near a stream, and many other individuals were observed at night on tree branches or on rocks at ca. 0–80 cm above the ground near streams in evergreen forest. This is a new record for Copia NR.

Odorrana nasica (Boulenger, 1903) (Fig. 4J): Two specimens were found at night on a tree branch near a waterfall, and many other individuals were observed at night on tree branches or on rocks at ca. 0–90 cm above the ground near waterfalls in evergreen forest. This is a new record for Copia NR.

Sylvirana guentheri (Boulenger, 1882) (Fig. 4K): Individuals were observed at night on the ground near pond edges and streams. The surrounding habitat was rice fields.

Sylvirana nigrovittata (Blyth, 1856) (Fig. 4L): Two specimens were found at night on the ground near a stream, and other individuals were observed at night on the ground, and on stones near streams or at the water edge in streams. The surrounding habitat was evergreen forest. The call concerts of this species were regularly heard in the evening.

Rhacophoridae

Chirixalus doriae Boulenger, 1893 (Fig. 4M): One specimen was found at night on a tree branch near a puddle, and five individuals were observed at night while sitting on leaves near puddles at ca. 30–80 cm above the ground. The surrounding habitat was mixed evergreen forest of small hardwoods, bamboo, and shrubs.

Gracixalus jinxiuensis (Hu, 1978) (Fig. 4N): Two specimens were found at night while sitting on leaves near a stream at ca. 1–2 m above the ground. The surrounding habitat was mixed evergreen forest of small hardwoods, bamboo, and shrubs. This is a new record for Son La Province.

Gracixalus quangi Rowley, Dau, Nguyen, Cao, and Nguyen, 2011 (Fig. 4O): Two specimens were found at night on leaves near a stream, and other individuals were observed at night while sitting on leaves near streams at ca. 0.8–1.5 m above the ground. The surrounding habitat was mixed evergreen forest of small hardwoods, bamboo, and shrubs.

Gracixalus truongi Tran, Pham, Le, Nguyen, Ziegler, and Pham, 2023 (Fig. 4P): Two specimens were found at night while sitting on leaves at ca. 1–1.5 m above the ground. The surrounding habitat was mixed evergreen forest of small hardwoods, bamboo, and shrubs in limestone mountains.

Kurixalus bisacculus (Taylor, 1962) (Fig. 4Q): Two specimens were found at night on the tree branches near puddles, and other individuals were observed at night while sitting on leaves or branches near streams or puddles at ca. 0.2–3.0 m above the ground. The surrounding habitat was cultivated land and mixed evergreen forest of small hardwoods, bamboo, and shrubs. This is a new record for Copia NR.

Polypedates megacephalus Hallowell, 1861 (Fig. 4R): Two specimens were found at night on the tree branches near a stream, and other individuals were observed at night while sitting on leaves or branches near streams, puddles, and ponds at ca. 0.3–2.5 m above the ground. The surrounding habitat was cultivated land and mixed evergreen forest of small hardwoods, bamboo, and shrubs. This is a new record for Copia NR.

Raorchestes parvulus (Boulenger, 1893) (Fig. 5A): Two specimens were found at night sitting on leaves near streams, and other individuals were observed on the same perch sites at ca. 1.5–4.0 m above the ground in evergreen forest. This is a new record for Copia NR.

Rhacophorus kio Ohler and Delorme, 2006 (Fig. 5B): Individuals were observed at night sitting on leaves near puddles at ca. 1–3 m above the ground in evergreen forest.



Fig. 5. Amphibian species recorded in Cobia Nature Reserve, Vietnam. (A) *Raorchestes parvulus*, (B) *Rhacophorus kio*, (C) *R. rhodopus*, (D) *Theloderma albopunctatum*, (E) *T. bicolor*, (F) *T. corticale*, (G) *T. gordonii*, (H) *Zhangixalus dorsovirens*, (I) *Z. feae*, and (J) *Tylotriton anguliceps*.

Rhacophorus rhodopus Liu and Hu, 1960 (Fig. 5C): One specimen was found at night sitting on leaves near puddles, and other individuals were observed at night while sitting on the same perch sites at ca. 2–3 m above the ground in evergreen forest.

Theloderma albopunctatum (Liu and Hu, 1962) (Fig. 5D): One specimen was found at night on edge of the waterhole and six individuals were observed at night at the same perch sites in evergreen forest.

Theloderma bicolor (Bourret, 1937) (Fig. 5E): One specimen was found at night on edge of a water filled tree hole in evergreen forest.

Theloderma corticale (Boulenger, 1903) (Fig. 5F): One specimen was found at night on edge of a water filled tree hole in evergreen forest.

Theloderma gordonii Taylor, 1962 (Fig. 5G): Two specimens were found at night on edge of a water filled tree hole in evergreen forest.

Zhangixalus dorsovirens (Bourret, 1937) (Fig. 5H): Two specimens were found at night on the tree branches near a stream in evergreen forest.

Zhangixalus feae (Boulenger, 1893) (Fig. 5I): Individuals were observed at night while sitting on tree branches or leaves near streams at 1–4 m above the ground in evergreen forest.

Caudata

Salamandridae

Tylototriton anguliceps Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes, and Nguyen, 2015 (Fig. 5J): Individuals were observed during the daytime underneath the carpet of fallen leaves near small streams in evergreen forest.

Reptilia

Squamata

Agamidae

Acanthosaura lepidogaster (Cuvier, 1829) (Fig. 6A): Individuals were observed while sitting on trees at ca. 1–2 m above the ground or while crossing a forest path in evergreen forest.

Calotes emma Gray, 1845 (Fig. 6B): One specimen was found in the morning on a forest path in evergreen forest. This is a new record for Copia NR.

Calotes versicolor (Daudin, 1802) (Fig. 6C): Individuals were observed during the daytime near cultivated lands or bushes in gardens.

Draco maculatus (Gray, 1845) (Fig. 6D): A road-killed individual was found on Road 108. The surrounding habitat was mixed evergreen forest of hardwoods and shrubs.

Pseudocalotes brevipes (Werner, 1904) (Fig. 6E): One specimen was found at night on a tree branch, and six individuals were observed at night while sitting on the same perch sites at ca. 1–2 m above the ground in evergreen forest.

Gekkonidae

Gekko reevesii (Gray, 1831) (Fig. 6F): Individuals were observed at night on limestone cliffs or large trees at ca. 1–6 m above the ground in evergreen forest.

Hemidactylus frenatus Duméril and Bibron, 1836 (Fig. 6G): Individuals were observed at night on a wall near a light bulb in a residential area.

Hemidactylus garnotii Duméril and Bibron, 1836 (Fig. 6H): Two specimens were found and other individuals were observed at night on limestone karst outcrops at 1–3 m above the ground in secondary forest.

Scincidae

Eutropis longicaudatus (Hallowell, 1857) (Fig. 6I): Individuals were observed during the daytime on the ground at road edges, and on shrubs near cultivated land.

Eutropis multifasciatus (Kuhl, 1820) (Fig. 6J): Individuals were observed during the daytime on the ground at road edges, and on shrubs near cultivated land. This is a new record for Copia NR.

Plestiodon cf. *tamdaoensis* (Bourret, 1937) (Fig. 6K): One specimen was found during the daytime on the ground at road edges near cultivated land. This is a new record for Copia NR.

Scincella devorator Darevsky, Orlov, and Ho, 2004 (Fig. 6L): One specimen was found in the afternoon on a forest path in evergreen forest.

Sphenomorphus indicus (Gray, 1853) (Fig. 6M): One specimen was found in the afternoon on a forest path and other individuals observed on the ground in evergreen forest.

Tropidophorus baviensis Bourret, 1939 (Fig. 6N): Two specimens were found under a carpet of fallen leaves, at a forest edge near evergreen forest.

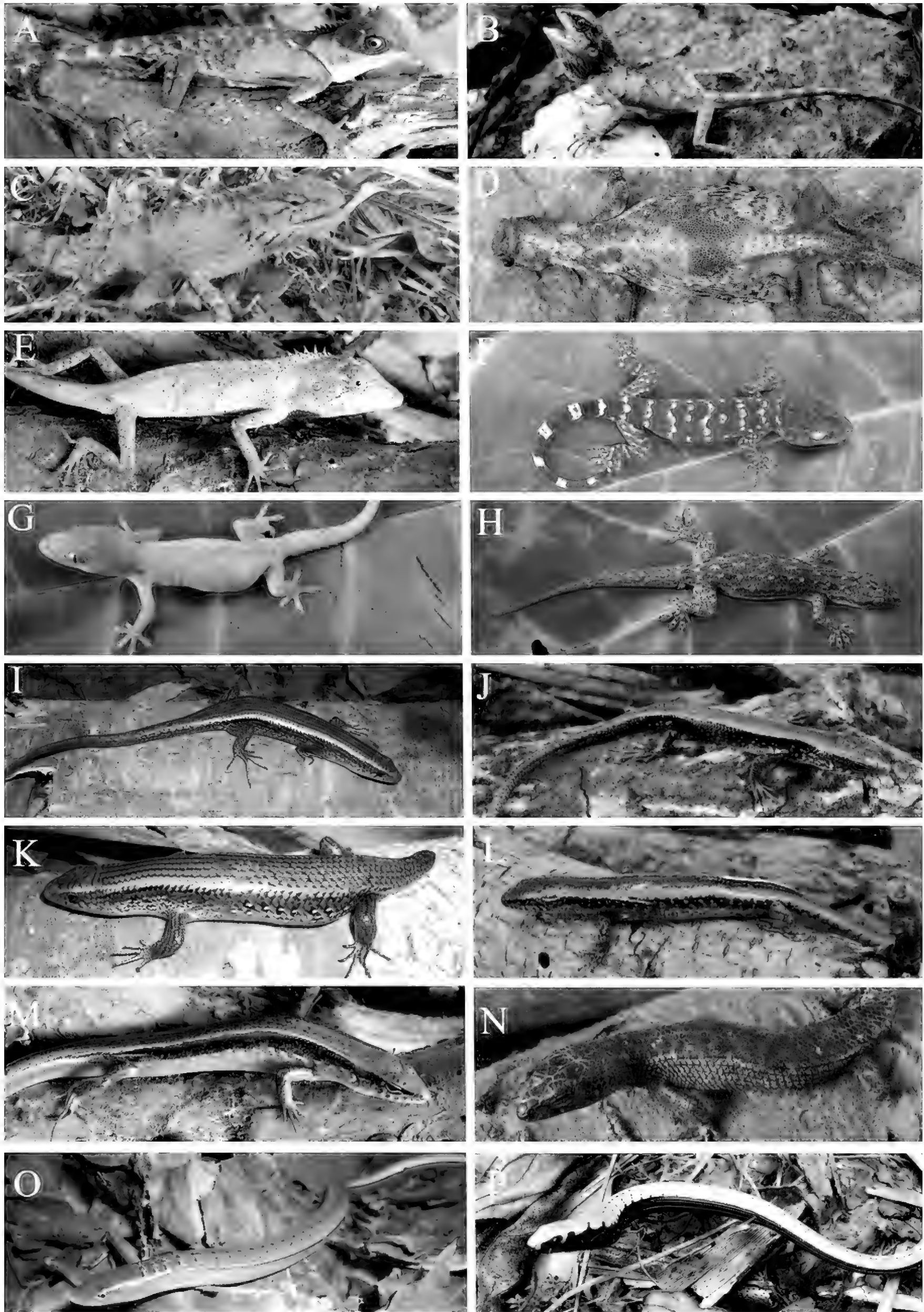


Fig. 6. Lizard species recorded in Copia Nature Reserve, Vietnam. (A) *Acanthosaura lepidogaster*, (B) *Calotes emma*, (C) *C. versicolor*, (D) *Draco maculatus*, (E) *Pseudocalotes brevipes*, (F) *Gekko reevesii*, (G) *Hemidactylus frenatus*, (H) *H. garnotii*, (I) *Eutropis longicaudatus*, (J) *E. multifasciatus*, (K) *Plestiodon* cf. *tamdaoensis*, (L) *Scincella devorator*, (M) *Sphenomorphus indicus*, (N) *Tropidophorus baviensis*, (O) *Dopasia harti*, and (P) *D. ludovici*.

Dopasia harti (Boulenger, 1899) (Fig. 6O): One specimen was found during the daytime on the ground at road edges near evergreen forest.

Dopasia ludovici (Mocquard, 1905) (Fig. 6P): Two specimens were found during the daytime on the ground at road edges near evergreen forest. This is a new record for Son La Province.

Typhlopidae

Indotyphlops braminus (Daudin, 1803) (Fig. 7A): Two specimens were found in the afternoon underneath a rotten plank near the forest edge.

Xenopeltidae

Xenopeltis unicolor Reinwardt, 1827 (Fig. 7B): Two individuals were observed at night on the ground near the forest edge.

Colubridae

Ahaetulla prasina (Boie, 1827) (Fig. 7C): Individuals were found at night on tree branches at ca. 1.0–2.5 m above the ground in secondary forest or on a fence near agricultural areas.

Boiga multomaculata (Boie, 1827) (Fig. 7D): Three individuals were observed at night on tree branches at ca. 1–3 m above the ground in evergreen forest.

Calamaria pavementata Duméril, Bibron, and Duméril, 1854 (Fig. 7E): One specimen was found at night on the ground while moving across a forest trail near evergreen forest. This is a new record for Copia NR.

Coelognathus radiatus (Boie, 1827) (Fig. 7F): Two individuals were observed in the afternoon while moving across a road near agricultural areas.

Dendrelaphis ngansonensis (Bourret, 1935) (Fig. 7G): One specimen was found during the daytime on the ground on a road edge near the evergreen forest. This is a new record for Copia NR.

Dendrelaphis pictus (Gmelin, 1789) (Fig. 7H): Two specimens were found during the daytime on tree branches at road edges near evergreen forest.

Elaphe moellendorffi (Boettger, 1886) (Fig. 7I): One individual was observed at 1805 h near the entrance of a cave at a forest edge. This is a new record for Copia NR.

Elaphe taeniura (Cope, 1861) (Fig. 7J): Two individuals were observed in the afternoon at the entrance of a cave near a forest edge and agricultural area. This is a new

record for Copia NR.

Euprepiophis mandarinus (Cantor, 1842) (Fig. 7K): One individual was observed at night on the ground near a stream in evergreen forest. This is a new record for Copia NR.

Gonyosoma coeruleum Liu, Hou, Lwin, Wang, and Rao, 2021 (Fig. 7L): One individual was observed at night on a tree branch near a stream in evergreen forest. Previous records of *Gonyosoma prasinum* in Copia NR by Pham et al. (2014) should be re-identified as *B. coeruleum* after Liu et al. (2021).

Gonyosoma frenatum (Gray, 1853) (Fig. 7M): A road-killed specimen was found in the morning on a road near evergreen forest.

Liopeltis frenata (Günther, 1858) (Fig. 7N): A road-killed specimen was found in the morning on a road near evergreen forest.

Lycodon fasciatus (Anderson, 1879) (Fig. 7O): One specimen was found at night on the ground while catching prey near a road, and five other individuals were observed at night on the ground near streams or forest trails in evergreen forest. This is a new record for Copia NR.

Lycodon futsingensis (Pope, 1928) (Fig. 7P): One specimen was found at night on the ground near a stream in evergreen forest. This is a new record for Copia NR.

Oligodon catenatus (Blyth, 1854) (Fig. 7Q): One specimen was found at night on the ground near a forest trail in evergreen forest, and a road-killed specimen was found on the road.

Oligodon fasciolatus (Günther, 1864) (Fig. 7R): One specimen was found at night on the ground in evergreen forest. This is a new record for Copia NR.

Oreocryptophis porphyraceus (Cantor, 1839) (Fig. 8A): One individual was observed at night on the ground while moving near a forest trail in evergreen forest.

Ptyas korros (Schlegel, 1837) (Fig. 8B): Two individuals were observed during the daytime on the ground at the roadside near a forest edge.

Ptyas multicincta (Roux, 1907) (Fig. 8C): One individual was observed at night on a tree branch in evergreen forest.

Sibynophis collaris (Gray, 1853) (Fig. 8D): One specimen was found during the daytime on the ground while moving across the road. This is a new record for Copia NR.



Fig. 7. Snake species recorded in Copia Nature Reserve, Vietnam. (A) *Indotyphlops braminus*, (B) *Xenopeltis unicolor*, (C) *Ahaetulla prasina*, (D) *Boiga multomaculata*, (E) *Calamaria pavementata*, (F) *Coelognathus radiatus*, (G) *Dendrelaphis ngansonensis*, (H) *D. pictus*, (I) *Elaphe moellendorffi*, (J) *Elaphe taeniura*, (K) *Euprepophis mandarinus*, (L) *Gonyosoma coeruleum*, (M) *G. frenatum*, (N) *Liopeltis frenata*, (O) *Lycodon fasciatus*, (P) *L. futsingensis*, (Q) *Oligodon catenatus*, and (R) *O. fasciolatus*.

Elapidae

Bungarus fasciatus (Schneider, 1801) (Fig. 8E): One individual was observed at night on the ground while moving across the road near secondary forest.

Bungarus wanghaotingi Pope, 1928 (Fig. 8F): Two individuals were observed near a stream in secondary forest. Previous records of *B. multicinctus* in Copia NR by Le et al. (2009) and in Vietnam by Nguyen et al. (2009) should be re-identified as *B. wanghaotingi* after Chen et al. (2021). Chen et al. (2021) stated that previous records of *B. multicinctus* in Vietnam and southern China should be re-identified as *B. wanghaotingi*. However, these species are difficult to distinguish morphologically. *Bungarus wanghaotingi* differs from *B. multicinctus* by having fewer light cross bands on the body and tail (20–31 and 7–11 versus 31–40 on the body and 9–17 on the tail in *B. multicinctus*, respectively). The specimens from Copia NR have 25–26 and seven light cross bands on the body and tail, respectively.

Naja atra Cantor, 1842 (Fig. 8G): Two individuals were observed during the daytime on the ground near bamboo bush in secondary forest.

Sinomicrurus macclellandi (Reinhardt, 1844) (Fig. 8H): One individual was observed at night on the ground while moving near a forest trail in evergreen forest.

Lamprophiidae

Psammodynastes pulverulentus (Boie, 1827) (Fig. 8I): One individual was observed at night on a tree branch in limestone forest. This is a new record for Copia NR.

Natricidae

Fowlea flavipunctatus (Hallwell, 1861) (Fig. 8J): An individual was observed in the afternoon near a pond. The surrounding habitat was secondary forest.

Hebius boulengeri (Gressitt, 1937) (Fig. 8K): Two specimens were found at night on the ground near a stream in evergreen forest. This is a new record for Copia NR.

Hebius chapaensis (Bourret, 1934) (Fig. 8L): One specimen was found at night in a stream in evergreen forest.

Rhabdophis subminiatus (Schlegel, 1837) (Fig. 8M): Three individuals were observed during the daytime on grass near the roadside in an agricultural cultivated area.

Trimerodytes percarinatus (Boulenger, 1899) (Fig. 8N): Two individuals were observed at night in streams in evergreen forest. This is a new record for Copia NR.

Pareatidae

Pareas carinatus (Boie, 1828) (Fig. 8O): One specimen was found at night while sitting on a tree branch in the secondary forest. This is a new record for Copia NR.

Pareas hamptoni (Boulenger, 1905) (Fig. 8P): One specimen was found at night while sitting on a tree branch, and many other individuals were observed at night on tree branches at ca. 0.6–1.5 m above the ground in evergreen forest.

Pareas margaritophorus (Jan, 1866) (Fig. 8Q): Two specimens were found at night while sitting on tree branches at ca. 0.6–1.0 m in evergreen forest.

Pseudoxenodontidae

Pseudoxenodon macrops (Blyth, 1854) (Fig. 8R): Two specimens were found in the morning while moving across a forest trail in evergreen forest.

Viperidae

Ovophis makazayazaya (Takahashi, 1922) (Fig. 9A): Two specimens were found at night while moving across a forest trail in evergreen forest. This is a new record for Son La Province.

Ovophis monticola (Günther, 1864) (Fig. 9B): Two specimens were found at night on the ground near streams in evergreen forest.

Trimeresurus albolabris Gray, 1842 (Fig. 9C): Individuals were observed during the daytime on tree branches at ca. 0.5–1.2 m above the ground near secondary forest and cultivated agricultural areas.

Testudines

Platysternidae

Platysternon megacephalum Gray, 1831 (Fig. 9D): Two individuals were observed at night under a rock in a stream in evergreen forest.

Testudinidae

Manouria impressa (Guenther, 1882) (Fig. 9E): One individual was observed during the daytime in a house of the local people.



Fig. 8. Snake and turtle species recorded in Copia Nature Reserve, Vietnam. (A) *Oreocryptophis porphyraceus*, (B) *Ptyas korros*, (C) *P. multicincta*, (D) *Sibynophis collaris*, (E) *Bungarus fasciatus*, (F) *B. wanghaotingi*, (G) *Naja atra*, (H) *Sinomicrurus macclellandi*, (I), *Psammodynastes pulverulentus*, (J) *Fowlea flavipunctatus*, (K) *Hebius boulengeri*, (L) *Hebius chapaensis*, (M) *Rhabdophis subminiatus*, (N) *Trimerodytes percarinatus*, (O) *Pareas carinatus*, (P) *P. hamptoni*, (Q) *P. margaritophorus*, and (R) *Pseudoxenodon macrops*.

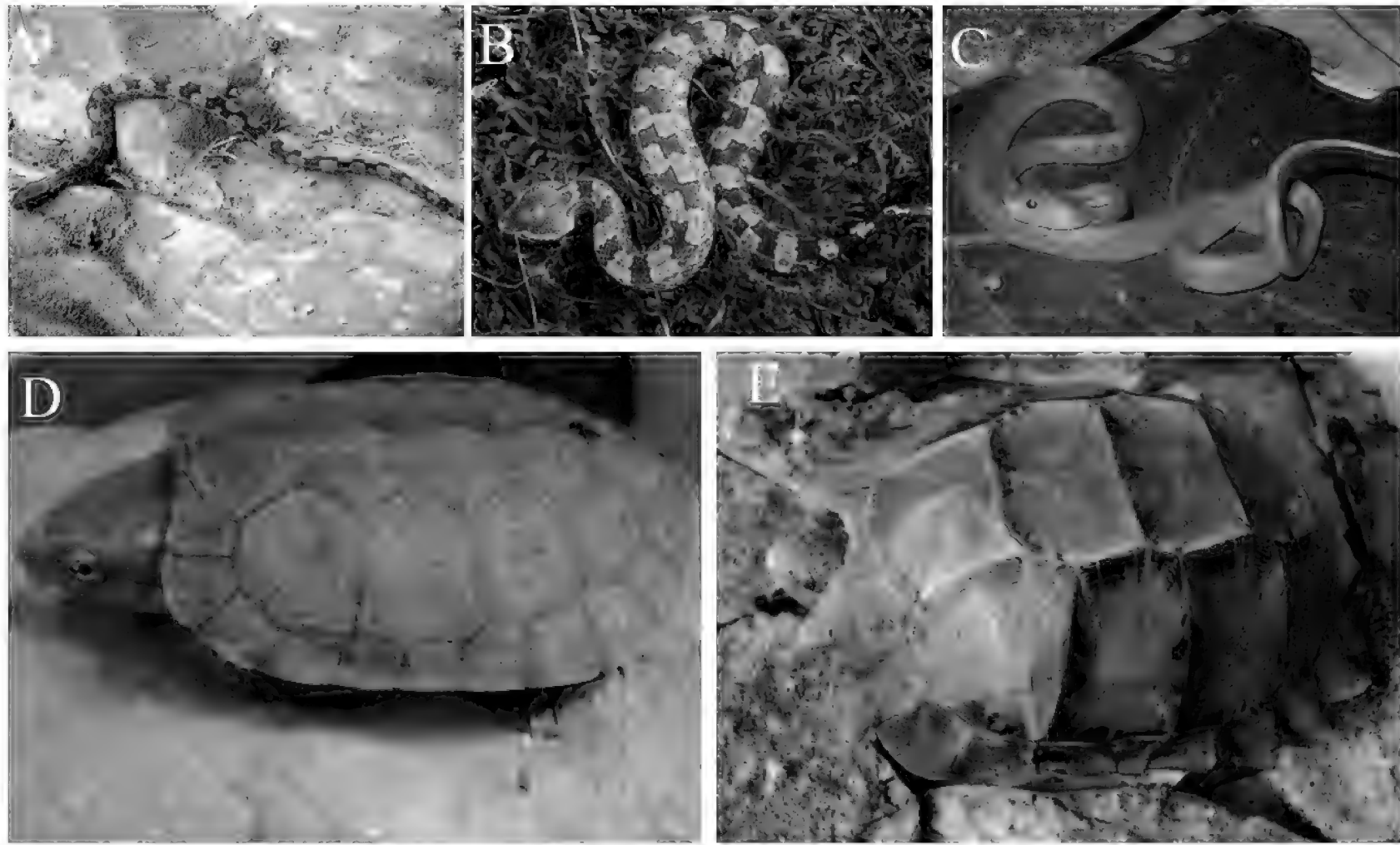


Fig. 9. Snake and turtle species recorded in Copia Nature Reserve, Vietnam. (A) *Ovophis makazayazaya*, (B) *O. monticola*, (C) *Trimeresurus albolabris*, (D) *Platysternon megacephalum*, and (E) *Manouria impressa*.

Discussion

Our new findings bring the number of amphibian and reptile species in Copia NR to 115, comprising 48 amphibian and 67 reptile species, and 25 of the species are new records for Copia NR while four are new records for Son La Province.

Among the six survey sites, the forest sites near Nong Vai and Hua Ty villages had the highest level of species richness, with 65 recorded species; followed by Huoi Pu forest with 52 species; Co Ma site with 37 species; Long He site with 36 species; and Pha Khuong site with 32 species (Table 2). The forest sites near Nong Vai and Hua Ty are in the core zone of the Copia NR with a large area of evergreen forest (>2,000 ha) and the habitat quality is relatively good. This explains why the number of recorded species is higher than those of other sites.

In terms of habitat preference, most of the amphibians and reptiles inhabit the evergreen forest (63 species, or 60% of the total recorded species), followed by disturbed secondary forest with 34 recorded species (32.38%), and agricultural areas with 24 recorded species (22.85%) (Table 2).

Concerning its herpetofaunal conservation status, Copia NR harbors many threatened species, including 17 species listed in the Red Data Book of Vietnam (Dang et al. 2007), with three species categorized as CR (*Boulengerophrys palpebralespinosa*, *Python bivittatus*, and *Ophiophagus hannah*); 10 species as EN (*Rhacophorus kio*, *Theloderma corticale*, *Zhangixalus feae*, *Varanus salvator*, *Coelognathus radiatus*, *Ptyas*

korros, *Ptyas mucosa*, *Bungarus fasciatus*, *Naja atra*, and *Platysternon megacephalum*); and four species as VU (*Elaphe moellendorffi*, *Euprepiophis mandarinus*, *Oreocryptophis porphyraceus*, and *Manouria impressa*). In addition, 13 species are listed in the IUCN Red List (IUCN 2023), with two species categorized as CR (*P. megacephalum* and *Mauremys sinensis*), one species as EN (*M. impressa*), eight species as VU (*Amolops vitreus*, *Odorrana graminea*, *Odorrana jingdongensis*, *Zhangixalus dorsovireidis*, *E. moellendorffi*, *Elaphe taeniura*, *N. atra*, and *O. hannah*), and two species as NT (*P. molurus* and *P. korros*). Eight species are listed in the Vietnam Governmental Decree No. 84/2021/ND-CP (2021), with two species included in Group IB (*O. hannah* and *P. megacephalum*) and six species in Group IIB (*Tylototriton anguliceps*, *V. salvator*, *P. molurus*, *P. mucosus*, *N. atra*, and *M. impressa*). Furthermore, eight species are listed in the CITES appendices, with one species included in Appendix I (*P. megacephalum*) and seven species in Appendix II (*T. anguliceps*, *V. salvator*, *P. molurus*, *P. mucosus*, *N. atra*, *O. hannah*, and *M. impressa*) (Table 2).

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Literature Cited

- Bain RH, Lathrop A, Murphy RW, Orlov NL, Ho CT. 2003. Cryptic species of a cascade frog from Southeast Asia: taxonomic revisions and descriptions of six new species. *American Museum Novitates* 3417: 1–60.
- Boulenger GA. 1893. Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea, dealing with the collection made in Pegu and the Karin Hills in 1887–88. *Annali del Museo Civico di Storia Naturale di Genova Serie 2* 13: 304–347.
- Bourret R. 1942. *Les Batraciens de l'Indochine*. Institut Océanographique de l'Indochine, Hanoi, Vietnam. 529 p.
- Chen ZN, Shi SC, Vogel G, Ding L, Shi JS. 2021. Multiple lines of evidence reveal a new species of Krait (Squamata, Elapidae, *Bungarus*) from Southwestern China and Northern Myanmar. *ZooKeys* 1025: 35–71.
- CITES. 2023. Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendices I, II, and III. Available: <https://cites.org/eng/app/appendices.php> [Accessed: 29 September 2023].
- Dang NT, Tran K, Dang HH, Nguyen C, Nguyen TN, Nguyen HY, Dang TD, Editors. 2007. *Vietnam Red Data Book. Part I. Animals*. Natural Science and Technology Publishing House, Hanoi, Vietnam. 515 p. [In Vietnamese]
- Fei L, Te CY, Jiang JP. 2012. *Colored Atlas of Chinese Amphibians and Their Distributions*. Sichuan Publishing House of Science and Technology, Chengdu, Sichuan, China. 620 p.
- Frost DR. 2023. Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, USA. Available: <https://amphibiansoftheworld.amnh.org/index.php> [Accessed: 29 September 2023].
- Hecht VL, Pham CT, Nguyen TT, Nguyen TQ, Bonkowski M, Ziegler T. 2013. First report on the herpetofauna of Tay Yen Tu Nature Reserve, northeastern Vietnam. *Biodiversity Journal* 4(4): 507–516.
- Inger RF, Orlov NL, Darevsky IS. 1999. Frogs of Vietnam: a report on new collections. *Fieldiana Zoology* 92: 1–46.
- IUCN. 2023. The IUCN Red List of Threatened Species. Version 2023.1. Available: <http://www.iucnredlist.org> [Accessed: 29 September 2023].
- Le TD, Nguyen TT, Nishikawa K, Nguyen LHS, Pham VA, Matsui M, Bernardes M, Nguyen QT. 2015a. A new species of *Tylototriton* Anderson, 1871 (Amphibia: Salamandridae) from Northern Indochina. *Current Herpetology* 34(1): 38–50.
- Le XH, Nguyen TTH, Nguyen TPL, Tran TD, Nguyen XT, Hoang VT, Tran HT, Ha VT, Nguyen QH, Nguyen TH, et al. 2009. An assessment of biological diversity of the Cópia Nature Reserve (Son La) and proposed solutions for conservation management. Unpublished report of the Institute of Ecology and Biological Resources, Hanoi, Vietnam. [In Vietnamese].
- Le TD, Pham VA, Nguyen LHS, Ziegler T, Nguyen QT. 2015b. First records of *Megophrys daweimontis* Rao and Yang, 1997 and *Amolops vitreus* (Bain, Stuart, and Orlov, 2006) (Anura: Megophryidae, Ranidae) from Vietnam. *Asian Herpetological Research* 6(1): 66–72.
- Liu S, Hou M, Lwin H-Y, Wang Q, Rao D. 2021. A new species of *Gonyosoma* Wagler, 1828 (Serpentes, Colubridae), previously confused with *G. prasinum* (Blyth, 1854). *Evolutionary Systematics* 5(1): 129–139.
- Mahony S, Kamei RG, Teeling EC, Biju SD. 2020. Taxonomic review of the Asian Horned Frogs (Amphibia: *Megophrys* Kuhl and Van Hasselt) of Northeast India and Bangladesh previously misidentified as *M. parva* (Boulenger), with descriptions of three new species. *Journal of Natural History* 54: 119–194.
- Nguyen SV, Ho CT, Nguyen TQ. 2009. *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main, Germany. 768 p.
- Nguyen QT, Pham VA, Nguyen LHS, Le DM, Ziegler T. 2015. First record of *Parafimbrios lao* Teynié, David, Lottier, Le, Vidal, and Nguyen, 2015 (Squamata: Xenodermatidae) from Vietnam. *Russian Journal of Herpetology* 22(4): 297–300.
- Pham VA, Le TD, Nguyen LHS, Ziegler T, Nguyen QT. 2014a. First records of *Leptolalax eos* Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler, and Dubois, 2011 and *Hylarana cubitalis* (Smith, 1917) (Anura: Megophryidae, Ranidae) from Vietnam. *Russian Journal of Herpetology* 21(3): 195–200.
- Pham VA, Le TD, Nguyen LHS, Ziegler T, Nguyen QT. 2015. New provincial records of skinks (Squamata: Scincidae) from northwestern Vietnam. *Biodiversity Data Journal* 3: e4284.
- Pham VA, Le TD, Pham TC, Nguyen LHS, Ziegler T, Nguyen QT. 2016. Two additional records of megophryid species, *Leptolalax minimus* (Taylor, 1962) and *Leptobrachium masatakasatoi* Matsui, 2013, for the herpetofauna of Vietnam. *Revue Suisse de Zoologie* 123(1): 43–47.
- Pham VA, Nguyen LHS, Nguyen QT. 2014b. New records of snakes (Squamata: Serpentes) from Son La Province, Vietnam. *Herpetology Notes* 7: 771–777.
- Pham VA, Nguyen LHS, Nguyen VT, Tran TTN, Nguyen QT. 2014c. New records of three frogs of the family Megophryidae (Amphibia: Anura) from Son La Province. *VNU Journal of Science* 30(1S): 1–6.
- Pham VA, Nguyen QT. 2018. Diversity of the genus *Theloderma* (Amphibia: Anura: Rhacophoridae) from Son La Province. *VNU Journal of Science* 34(1): 48–54.
- Pham VA, Nguyen QT, Pham TC, Sung BN, Le DM, Toulor V, Ziegler T. 2022. New records of amphibians from Son La Province, Vietnam. *Herpetology Notes* 15: 169–178.
- Pham VA, Nguyen QT, Ziegler T, Nguyen TT. 2017.

- New records of tree frogs (Anura: Rhacophoridae: *Rhacophorus*) from Son La Province, Vietnam. *Herpetology Notes* 10: 379–386.
- Pham VA, Nguyen TBN, Nguyen TM, Nguyen LHS, Nguyen QT. 2013. New records of snakes (Squamata: Serpentes) from Son La Province. Pp. 466–472 In: *Proceedings of the 5th National Scientific Conference on Ecology and Biological Resources*. Vinh University Publishing House, Vinh City, Nghe An Province, Vietnam.
- Pham VA, Nguyen VT, Nguyen LHS, Nguyen QT. 2012. First records of *Nanorana aenea* (Smith, 1922) and *Gracixalus quangi* Rowley, Dau, Nguyen, Cao, and Nguyen, 2011 (Amphibia: Anura) from Son La Province. Pp. 34–39. In: *Proceedings of the Second National Scientific Workshop on Amphibians and Reptiles of Vietnam*. Agricultural Publishing House, Hanoi City, Vietnam.
- Pham VA, Pham TC, Doan DL, Ziegler T, Nguyen QT. 2019. New records of megophryids (Amphibia: Anura: Megophryidae) from Son La Province, Vietnam. *Biodiversity Data Journal* 7: e39140.
- Pham VA, Tu HV, Nguyen VT, Ziegler T, Nguyen TQ. 2018. New records and an updated list of lizards from Son La Province, Vietnam. *Herpetology Notes* 11: 209–216.
- Pham AV, Ziegler T, Nguyen TQ. 2020. New records and an updated list of snakes from Son La Province, Vietnam. *Biodiversity Data Journal* 8: e52779.
- Simmons JE. 2002. Herpetological collecting and collections management, revised edition. *Herpetological Circular* 31: 1–153.
- Smith MA. 1935. *The Fauna of British India, including Ceylon and Burma. Reptilia and Amphibia. Volume 2-Sauria*. Taylor and Francis, London, United Kingdom. 440 p.
- Smith MA. 1943. *The Fauna of British India, Ceylon and Burma, including the Whole of the Indo-Chinese Subregion. Reptilia and Amphibia. Volume 3-Serpentes*. Taylor and Francis, London, United Kingdom. 583 p.
- Taylor EH. 1962. The amphibian fauna of Thailand. *University of Kansas Science Bulletin* 43: 265–599.
- The Government of Vietnam. 2021. *The Governmental Decree No. 84/2021/NĐ-CP, Dated on 22th September 2021, on Management of Endangered, Precious, and Rare Species of Wild Plants and Animals*. Government of Vietnam, Hanoi, Vietnam. 13 p.
- The People's Committee of Son La Province. 2019. *Geography of Son La Province*. Truth National Political Publishing House, Tuyên Quang, Vietnam. 931 p.
- Tran TT, Pham VA, Le DM, Nguyen NH, Ziegler T, Pham TC. 2023. A new species of *Gracixalus* (Anura, Rhacophoridae) from northwestern Vietnam. *ZooKeys* 1153: 15–35.
- Uetz P, Freed P, Aguilar R, Hošek J, Editors. 2023. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 29 September 2023].
- Ziegler T, Nguyen QT, Pham TC, Nguyen TT, Pham VA, Nguyen TT, Schingen VM, Nguyen TT, Le DM. 2019. Three new species of the snake genus *Achalinus* from Vietnam (Squamata: Xenodermatidae). *Zootaxa* 4590(2): 249–269.



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The methodology for rearing the Fire-bellied Toad (*Bombina bombina*) from protected, small, isolated (but degraded) habitats in its northern distribution range

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Abstract.—The Fire-bellied Toad (*Bombina bombina* Linnaeus, 1761) is a vulnerable and protected species in Europe, where it is suppressed in small, isolated populations in its northern distribution range. The main cause of *B. bombina* population declines in this region is the loss of suitable habitats due to either anthropogenic factors or natural succession. Recently, very hot summers with prolonged dry and very heated periods have contributed to the declines of *B. bombina* populations on a very large scale. Therefore, it is important to preserve the natural, although small, populations of *B. bombina* to save the gene pool of the rare northern populations for the future, which is essential for conservation breeding, research, and outreach with this species. This study provides the rearing methodology, growth rates, and sexual dimorphism of protected *B. bombina* individuals in their first year.

Keywords. Anura, endangered species, conservation, sexual dimorphism, morphometrics, Natura 2000

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Introduction

Interest in species conservation is growing each year, and amphibian species are one of the most endangered groups of vertebrates worldwide (Kurnaz and Kutrup 2019; Stuart et al. 2008; Wake and Vredenburg 2008). The Fire-bellied Toad (*Bombina bombina* Linnaeus, 1761) is one of these vulnerable amphibian species that is distributed mainly in Central and Eastern Europe (Nicoara and Nicoara 2007; Sillero et al. 2014).

Bombina bombina is a tailless amphibian. The body of this frog is flat, and its length varies from approximately 40 to 55 mm. The back of *B. bombina* is dark brown or greyish, and the underbelly is mottled with wide red and orange spots that they use to scare away predators (Rimšaitė 2021). The most suitable habitats for *B. bombina* are in lowlands such as shallow fish-free ponds with plenty of sunlight, or shallow edges of larger water bodies (Rimšaitė 2021; Schröder et al. 2012). This species can also inhabit swamps, flooded

ditches, quarries, and peat bogs (Chikhlyayev and Ruchin 2021). *Bombina bombina* breeds in stagnant, good-quality water with natural eutrophic conditions (Kinne et al. 2006). According to research conducted in Latvia, *B. bombina* calling males were found in small (<0.5 ha) and medium (0.5–10.0 ha) sized lentic waterbodies and ditches (Čeirans et al. 2020).

The northern range of the distribution of *B. bombina* in Europe is within northern Lithuania and the south-eastern parts of Latvia (Kuzmin et al. 2008; Pupina and Pupins 2008, 2009). This species is protected in both countries (Berzins 2003; Rimšaitė 2021). Although the range of *B. bombina* covers all Lithuanian territory, it has a very fragmented distribution. In the territory of Lithuania, approximately 20,000–50,000 *B. bombina* individuals can be found, but there are only a few suppressed populations in the northeastern and western parts of Lithuania. Most of these populations are small, consisting of 10–20 adult individuals (Ivinskis and Rimšaitė 2011; Rimšaitė 2021).

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At the beginning of the 20th century, *B. bombina* was not classified as a rare species in Lithuania (Fedorowicz 1918). Today it is classified as a Near Threatened species, and it is included in the Red Data Book of Lithuania (Rimšaitė 2021). A similar situation is seen in Latvia, where *B. bombina* populations are very small and isolated (Pupins and Pupina 2012). In most of these populations, no more than 20 vocalizing males were recorded (Pupina 2011; Pupina and Pupins 2007). In Latvia, this species is included in the Red Book of Latvia and assigned to the first endangered species category (Berzins 2003). This is the most recent information described in the literature, but the current situation could be even worse. Furthermore, it is also listed as a protected species in Appendix II of the Bern Convention and Annexes II and IV of the EU Habitats Directive. *Bombina bombina* is included in the IUCN Red List of Threatened Species, where its most recent assessment was conducted in 2008 and it was listed as Least Concern (IUCN 2023).

The main causes of declining *B. bombina* populations in its northern distribution range are the loss of suitable habitats due to either anthropogenic factors or natural succession (Pupina et al. 2018; Pupins and Pupina 2012; Tytar et al. 2018). In some water bodies, populations of *B. bombina* are reduced by the appearance of the highly invasive, predatory fish species *Perccottus glenii* Dybowski, 1877 (Pupina and Pupins 2008). In Lithuania, the main drivers of *B. bombina* decline are changes in land use within the habitats of the species, and climate change leading to prolonged heated periods in the spring and summer (Rimšaitė 2021). When small ponds dry up, the tadpoles or even adult individuals often die. This can represent a crucial loss for the small, isolated *B. bombina* populations that are at a very high risk for the loss of genetic variation through genetic drift and inbreeding (Frankham and Ralls 1998). Furthermore, strong shifts in the sex ratios in several isolated populations have been noted, as they have become more isolated during such habitat degradation. Some of these populations became male dominated, while others became female dominated, possibly resulting from the very small numbers of *B. bombina* individuals in these populations. Therefore, it is important to save these natural, although small, populations of *B. bombina* in order to stabilize their sex ratio and re-establish them in more suitable habitats for their continuing occurrence within their northern distribution range. To achieve this, it is important to use *ex situ* methods such as rearing *B. bombina* individuals in the laboratory and releasing juveniles back into their natural habitats, as they will have a higher chance of survival compared to other stages of development. It is also important to choose suitable ponds for the release of *B. bombina* individuals, where their survival and breeding *in situ* in their natural environment would be ensured. Releasing individuals into nature in an equal sex ratio contributes to better survival of the population.

The aim of the study was to develop an effective methodology for the rearing of collected *B. bombina* spawn from degraded habitats of the small, isolated populations. Several spawn of *B. bombina* individuals were collected from degraded habitats in three locations in Lithuania between 2017 and 2021. Collected spawn was incubated, and the hatched tadpoles were grown and released into more suitable, protected habitats near the initial collection sites. In this article, we provide the rearing methodology, growth rates, and sexual dimorphism of reared *B. bombina* individuals. This methodology of *B. bombina* egg-rearing until the juvenile stage may contribute to saving the small, isolated gene pools of *B. bombina* populations for the future at the northern edge of its species distribution.

Materials and Methods

Collection of *Bombina bombina* Spawn

Spawn of *B. bombina* was collected from naturally degraded ponds in four Natura 2000 sites in Lithuania: Juodabalė Zoological Reserve (LTLAZ0010); Drapalai village surroundings (LTDRU0004); Kučiuliskė village surroundings (LTLAZ0001); and Margiai village surroundings (LTLAZ0035) (Table 1). Natura 2000 is an ecological network of rare and threatened species protection sites, which stretches across all 27 European Union countries. Spawn was collected during the end of spring and beginning of summer (May–June), when water temperatures reached 13–14 °C, in 2017, 2018, 2020, and 2021. The year 2019 was not included in the study, because that spring was very hot and dry which caused the total drying out of the *B. bombina* spawn. The spawn of eggs was carefully collected from flooded plants at the earliest stage of their development and placed in a 10 L bucket half-filled with pond water. Water from the ponds was taken along with the resident microfauna so that the tadpoles could feed in the early stages. The buckets with eggs were kept outdoors to protect them from strong temperature fluctuations, and at 1–2 days after collection, they were delivered to the Lithuanian Zoological Gardens (Kaunas, Lithuania).

Incubation of Spawn

The delivered spawn with water was very carefully transferred to 60 L plastic containers (Fig. 1A) that were filled with water to three-fourths capacity. The water pH was 7.6–7.8, and total water hardness was 2.6–2.8 mmol/L. In the laboratory, the initial water temperature was maintained similar to outdoors (13–14 °C), and within 5 days the temperature was gradually increased to 24–25 °C and maintained at that level for the remainder of the study. The temperature was increased gradually to avoid thermal shock to the eggs. Overall, the average water temperature was maintained

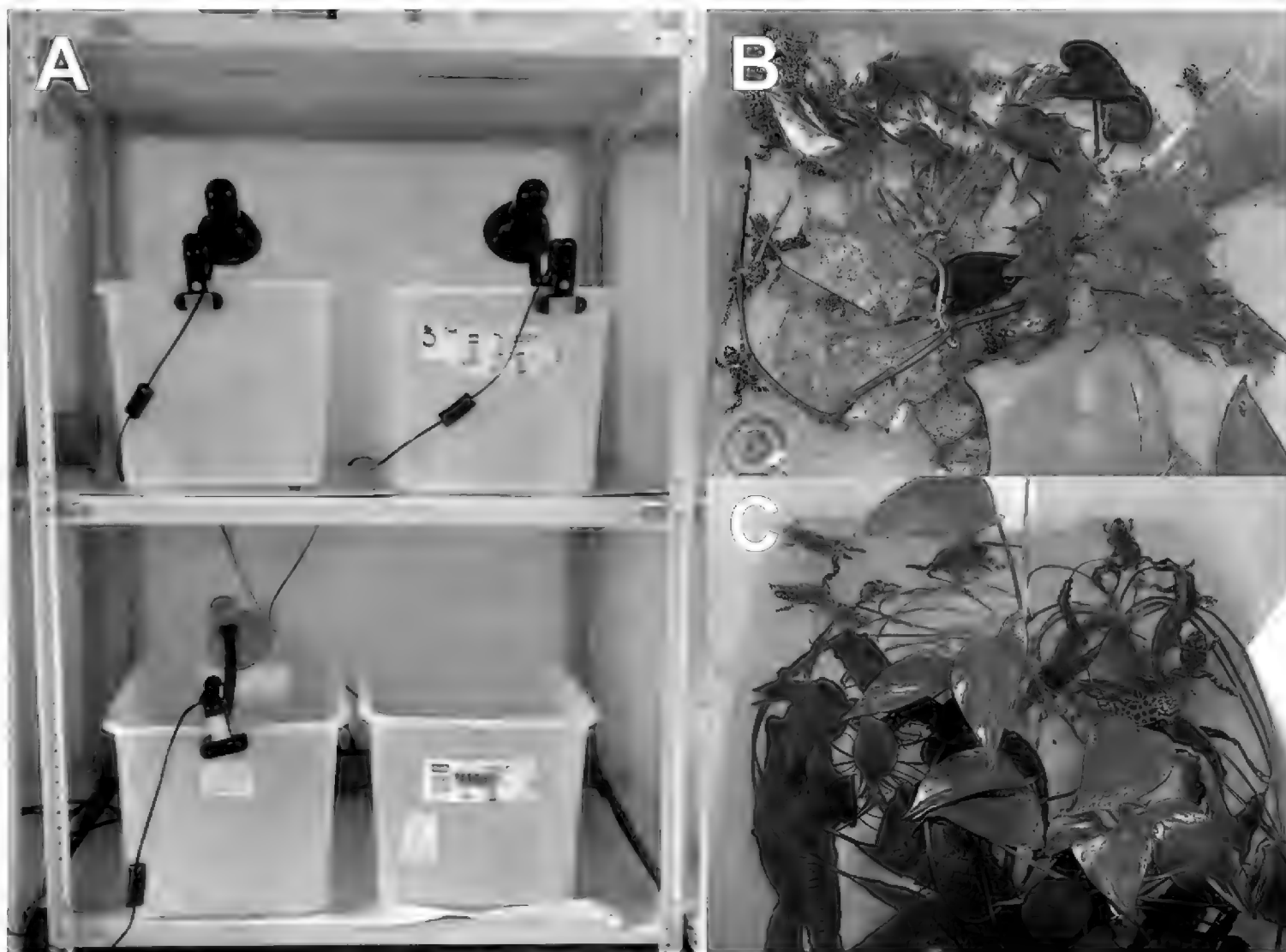


Fig. 1. *Bombina bombina* rearing system. (A) Plastic rearing tanks. (B) Three-month-old *B. bombina* individuals. (C) Eight-month-old *B. bombina* individuals.

at 21.0 ± 2.3 °C during all incubation periods. Before filling the containers, they were washed with antiseptic and rinsed thoroughly with hot water. The lids of containers were perforated with holes. The larvae hatched in 5–7 days.

Rearing during the Larval Period

After hatching, the *B. bombina* tadpoles were kept in 60 L plastic containers with dimensions of 58 x 35 x 30 cm. Every day, dirt was removed from the bottom with a sieve, and one-fifth of the water was drained, and fresh tap water was added slowly. The water with larvae was moved as little as possible. The water pH was 7.6–7.8, and total water hardness was 2.6–2.8 mmol/L. No water aeration or filtration was used. The average water temperature in the containers was maintained at 23.8 ± 1.1 °C during all metamorphosis stages. We evaluated the embryonic development according to the stages described by Gosner (1960), and the 23–25 stage of *B. bombina* development was reached in 3–5 days. At weekly intervals up to 3 months of age, the juveniles were grouped by their stage of development because their rate of development varied. Before metamorphosis was complete, when the tadpoles entered the 4-leg stage, they needed a place to climb, rest, and put their head above the water (i.e., land). They were transferred to 35 L plastic tanks with a water level of about 5–7 cm and parts of land without water. The tanks were placed at an angle of about 35–

45°. Dried oak leaves, live plants, stones, and other materials were placed in the water to make it easier for the juveniles to reach the land. The tanks were covered with dense gauze of 2 x 2 mm mesh size held in place with a rubber band because the juveniles could escape. Lighting was provided by 28W incandescent lamps and REPTI PLANET Repti UVB 2.0 13W (Czech Republic) lamps installed 20 cm above the tanks. The UVB-containing lamps were turned on every day for about 4 hours. UVB rays stimulate the production of vitamin D and calcium uptake (Michaels et al. 2015). The length of the photoperiod was similar to the natural rhythm of day and night, and it was adjusted according to the season.

During metamorphosis, it was important to keep enough microfauna in the water for the tadpoles to feed upon. Microfauna was collected along with the water during egg collection. Tree leaves that began to decay were added to the water so that microorganisms (such as *Paramecium* genus) could begin to proliferate. Dry oak leaves were added to the water due to their disinfectant properties and soft leaves of oak, willow, apple, and pear trees, along with blanched nettles, provided more food for the tadpoles. At the beginning, they were also fed daily with dry feed for fish fry Vipan Baby (Sera, Germany), and later with Bio-vit (Tropical, Poland).

The full larval development period of *B. bombina* lasted for about 45–65 days. Metamorphosis was completed when the juveniles completely lost their tail and had four fully grown legs.

Rearing and releasing Fire-bellied Toads (*Bombina bombina*)

Table 1. The locations (as the codes for LT Habitat Protection Important Territories) of *B. bombina* spawn collection and where reared individuals were released during the study period (2017–2022).

Collection date	Locations of collected spawns	Release date	Locations of release
2017 06 29	LTDRU0004	2018 06 22	LTLAZ0010
2018 05 04	LTLAZ0010	2019 06 14	LTLAZ0010
2018 05 15			LTLAZ0010
2020 05 30			LTLAZ0001
2020 06 02	LTLAZ0010	2021 06 11	LTLAZ0001
	LTLAZ0010		LTDRU0004
2021 05 13	LTLAZ0001	2022 06 17	LTLAZ0035
	LTDRU0004		

Post-metamorphosis Rearing

After they had completed metamorphosis, the 3-month-old juveniles were transferred to 60 L plastic tanks (Fig. 1B). The juveniles were grouped into three tanks according to size, in order to reduce interspecific competition, with 10 to 15 juveniles in each tank. The smallest juveniles were transferred to 10 L tanks, with a lot of vegetation (*Epipremnum* spp., *Anubias* spp., and *Cryptocoryne* spp.) to allow the juveniles to get out of the water. In these tanks, the water depth was about 2–4 cm. All the water in the tanks was changed at least twice a week, or more often if necessary. The average water temperature in the containers was maintained at 23.8 ± 0.6 °C during all post-metamorphosis periods. During post-metamorphosis rearing in the tanks, UVB-containing lamps were turned on every day for about 4 hours.

The juveniles were fed daily with an amount of food that corresponded to about 10–12% of their body weight. The first food source given to juveniles was live fruit flies (*Drosophilidae*), which were added to the rearing tank. House Crickets (1- to 3-day-old *Acheta domesticus*) and Two Spotted Crickets (1- to 5-day-old *Gryllus bimaculatus*) were given to very small and weak individuals. From 5 to 7 flies were provided for each juvenile, and the uneaten fruit flies were fed on carrot slices, cabbage leaves, etc. Vitamins and minerals were dusted on the fruit flies twice a week. Small crickets (*Gryllus* sp.) were added to the diet of one-month-old juveniles. Live mosquito larvae (*Chironomus* sp.) were added to the diet of juveniles at 6–7 weeks old. From the age of 2 months, juveniles were fed Turkestan Cockroaches (*Shelfordella tartara*). All feed was provided live.

Wintering Period of Rearing Juveniles

The overwintering period was carried out in the lab because the seasonal temperature variation is important for the development and phenology of temperate

amphibians, especially their reproductive cycles. Five-month-old *B. bombina* juveniles were prepared for wintering. Over a period of two weeks, the water temperature was gradually reduced to 10 °C and the photoperiod was reduced to 5 hours. The amount and caloric content of the food was also reduced gradually by 20% per day for five days in a row, and starting at two days before the wintering period, juveniles were no longer fed at all. This was done to ensure that the guts of overwintering toads were empty, otherwise the food inside would rot. Plastic tanks (15 L) with moist 5.5–6.5 pH neutralized peat (DURPETA, Lithuania) and softened dried oak leaves were prepared for the wintering of *B. bombina* juveniles. This substrate was placed in tanks at a depth of 5–7 cm. Ten to 15 juveniles were placed in the tanks for wintering. The wintering tanks were transferred to a wintering laboratory where the water temperature was maintained at 6–8 °C and checked daily. The wintering laboratory was locked, and the results of the temperature sensors were observed without entering the laboratory, in order to avoid causing any additional sounds. The laboratory was lit by a faint blue light. The condition of the juveniles was checked once a month and the *B. bombina* juveniles wintered for 12 weeks.

Post-wintering Period of Rearing Juveniles

At the end of wintering, the juveniles (now six months old) were transferred to the laboratory, where the temperature was 10 °C. After wintering, when the temperature reached at least 15 °C, the *B. bombina* juveniles were fed minimally with fruit flies. Later, the amount and caloric content of the food was gradually increased. Over one week, the water temperature was gradually raised to 17 °C. The photoperiod was extended to 8 hours. The juveniles were kept in plastic tanks for two more months until they reached 8 months of age (Fig. 1C), and then 20 individuals were placed

into each 100-liter aquarium with a 2–3 cm thick layer of gravel (2–5 mm diameter). The water level in the aquariums was about 10 cm. On the bottom of the aquariums, the substrate consisted of stones and other items, and artificial or live plants were added. The aquariums were equipped with automatic thermostats and filters with air compressors. Above the tanks were lighting fixtures with 28 W incandescent lamps and UVB 2.0 lamps. After full recovery, the average water temperature in the containers was maintained at 23.7 ± 0.5 °C during all post-wintering periods. Each time the water was changed, its level was raised by 1–2 cm to a final depth of 17–22 cm.

After recovery from wintering, all juveniles were divided into three equal groups according to weight to avoid interspecific competition. The separate groups were not mixed until their sex was determined, and they were released back into the wild.

Juvenile Rearing in Outdoor Tanks

When the maximum outdoor air temperature reached approximately 21–23 °C during the day, *B. bombina* individuals were moved outside. They were placed into 1.5 m² tanks covered with a fine net. In rainy weather, the tanks were covered with lids that had air vents. The bottoms of the tanks were covered with pebbles, and water plants were planted under the water. On cold nights (when the outside temperature dropped below 15–18 °C), the thermostats were switched on. In general, animals that will be reintroduced into nature must have as little contact with humans as possible. For this reason, the *B. bombina* individuals were not exhibited to visitors of the Lithuanian Zoological Gardens where they were reared.

Environmental Conditions and Promotion of Natural Behavior during Post-metamorphosis

After metamorphosis, the *B. bombina* juveniles lived in a more sterile environment than they would have experienced in nature. However, the soil, stones, oak leaves, live aquatic plants, and other natural materials provided hiding places and created an environment similar to nature. The juveniles would try to hide when they felt threatened.

Another even stronger way to promote the natural behaviour of the juveniles was to let them search for live food, i.e., to hunt for it. As mentioned above, the diet included live fruit flies, mosquito larvae, various cockroaches, and crickets. Competitive relations emerged between the *B. bombina* juveniles. In adaptation tanks, the juveniles were placed in a small area similar to their natural environment. There *B. bombina* juveniles could swim freely, and climb over the branches, rocks, or plants which were above the water.

Sex Determination and Morphometric Measurements

The sex of *B. bombina* individuals was determined as they matured (at 9 months old, after the full metamorphosis). In addition to sex identification by nuptial pads, croaking males were noted to confirm their sex. Moreover, some of the females spawned before their sex had been determined.

Total body weight and the two morphometric parameters of body (snout-vent) length (SVL) and sacral width (SW) were measured for the reared *B. bombina* individuals before they were released into the wild. Morphometric measurements were taken using a digital calliper (Carbon Fiber Composites, model CTCF1506, China; resolution 0.1 mm, accuracy ± 0.1 mm). The 1 mm margin of error in length and width measurements was permissible in order to avoid traumatizing the animals. The weight of juveniles was measured using scales (Romansas, model KB, Lithuania; accuracy 0.01 g and error ± 0.1 g).

Transporting the Animals and their Release into Nature

At 9 months of age, depending on the weather conditions, the *B. bombina* juveniles were handed over to the workers responsible for the protected areas for release into the wild. Juveniles were not fed for one day prior to being released into the wild. In the evening before release, juveniles were brought from the adaptation tanks and kept 2 degrees cooler. It was very difficult to predict the outdoor air temperature on the following day, and if the juveniles are abundantly fed and the air temperature cools suddenly, the feed can begin to spoil and kill the juveniles. In the morning, 10–15 *B. bombina* juveniles were placed in pre-made 15 L plastic boxes. The bottoms of the plastic boxes were covered with a 2 cm thick layer of moss (*Sphagnum* sp.) and a small amount of water to minimize the stress of the juveniles during transportation. Juveniles were released in pre-arranged Habitat Protection Important Territories (HPIT) of the Natura 2000 network territories in Lithuania (Table 1). The number of *B. bombina* juveniles to be released into each water body was arranged with the responsible personnel of the protected areas before the date of release.

Statistical Analyses

For collating the data, all measured juveniles were divided into two groups according to their gender, which was derived from their sexual behavior. The gender effects on the weight and measurements of snout-vent length (SVL) and sacral width (SW) of juveniles were estimated by two-way ANOVA, also including a year factor and their interaction. In all three cases, the

model residuals did not significantly deviate from a normal distribution (Shapiro-Wilk's tests: $p > 0.05$). The analyses were performed using STATISTICA 12.0 software. The significance level of $p < 0.05$ was specified for all statistical analyses *a priori*.

Results

Survival

In total, 229 tadpoles developed from the collected spawn during the study period. The proportion surviving to successful metamorphosis and the overall percentage of specimens that reached the adult stages were very high at 93.2 and 92.4%, respectively (Table 2). However, there were some considerable differences in the metamorphosis success of tadpoles between the years. The first two years showed the lowest survival rate, with very low numbers in 2018. That year tadpoles were reared in tanks with water filters, which made an effect of running water, while in later years the hatched tadpoles were grown in still water. After methodical changes in tadpole rearing, the success rate increased remarkably (Table 2).

Growth and Sexual Dimorphism

In nine months, the reared *B. bombina* individuals reached 38.9 ± 2.5 mm in body length, 22.7 ± 3.3 mm in body width, and they weighed 7.4 ± 1.3 g. The total body weight, length, and width indicating the growth of juveniles varied between males and females in the different years (Table 3). The available data allow for statistical testing of a simple hypothesis. Significant differences were observed in all three measurements between males and females, and females were bigger (Table 4). However, only the SVL and SW measurements varied significantly between the different years, while the comparison of different study years did not reveal any difference in juvenile weight (Table 4).

A retrospective comparison of the reared *B. bombina* juveniles revealed gender differences in their weight. Each year, the reared juveniles were grouped into three equal groups according to their total body weight after six months (Table 5). The sexing of the adults showed that the first juvenile weight group included almost 95% of the females. While the third group included almost entirely (90%) males. Gender was distributed almost equally in the middle group, at 43% male and 57% female. Overall, the results showed that the gender of six-month-old juveniles could be ascertained by separating them into three groups according to size even before they had truly matured. In this case, the gender could be assigned with more than 90% accuracy within the first (largest) and last (smallest) groups of separated juveniles (Table 5).

Discussion

Bombina bombina is considered to be of Least Concern globally (IUCN 2023), but it is also important to prevent local extinction. This species is distinguished as the targeted protected species in 31 Habitat Protection Important Territory (HPIT) areas of the Natura 2000 network territories in Lithuania (SRIS 2023). However, the significant decline of small, highly fragmented *B. bombina* populations was recently observed within the territory of Meteliai HPIT of the Natura 2000 network. In recent years, *B. bombina* individuals, and especially their spawn, have been dying due to prolonged late spring or summer droughts, during which the spawn laid in their usual reproduction habitats have dried up. *Bombina bombina* females usually spawn in shallow ponds on the stems of vertical water plants (Rimšaitė 2021). Each year, natural ponds are full of water at the end of spring, but then the water level drops suddenly, and the eggs die before they can hatch; and even if some of the tadpoles do hatch, they die as the water level drops.

Furthermore, fragmented *B. bombina* populations are declining because of accelerated natural succession, after remarkable changes in surrounding land use have occurred. In recent years, small water ponds that were once suitable for *B. bombina* breeding have become overgrown with grass, ponds are becoming shady, or they are being deepened for the development of extensive fisheries which cause dramatic habitat changes and fragmentation (Rimšaitė 2021). Moreover, the rapidly decreasing number of small farms at the country scale is leading to the destruction of many small ponds, which had been used as the drinking water source for farm animals and were also very suitable habitats for *B. bombina* individuals to thrive (Rimšaitė 2021). As a result, many *B. bombina* habitats with previously known populations are disappearing. Therefore, to protect the remaining small and protected *B. bombina* populations, our aim was to create a methodology for the collection and incubation of spawn, and post-metamorphosis tadpole rearing based on the environmental conditions where their development is highly threatened.

Spawn of *B. bombina* was collected and the subsequently reared individuals were released in the four HPITs (Table 1). *Bombina bombina* spawn was collected from specific water ponds where they had no chance for survival, while the reared specimens were released to either a different HPIT or the same HPIT, but in different watercourses with a good state for maintaining viable *B. bombina* populations. These watercourses are deep enough for *B. bombina* to successfully survive and reproduce, they do not dry up during hot spring or summer months, and do not become overgrown with grass. The release of *B. bombina* individuals in suitable water ponds will increase natural local populations of the species in the Natura 2000 protected sites. By increasing the *B. bombina* populations in these areas, the species has

Table 2. Numbers of developed *B. bombina* tadpoles, individuals after metamorphosis, and individuals released to water ponds from 2017–2022.

Egg collection year	Number of developed tadpoles	Number of individuals surviving after metamorphosis	Percentage of individuals surviving after metamorphosis	Release year	Number of individuals released to ponds	Percentage of overall survival of individuals
2017	69	64	92.8 %	2018	61	88.4 %
2018	56	31	55.4 %	2019	31	55.4 %
2020	70	70	100.0 %	2021	70	100.0 %
2021	73	67	91.8 %	2022	67	91.8 %
Total	268	232	93.2 %	–	229	92.4 %

Table 3. Numbers of male and female *B. bombina* juveniles and their mean weight values, lengths and widths from 2018–2022. Note: n – number of individuals; F – female; M – male; SD – Standard deviation

Year	n		Weight		Length		Width	
	F	M	F	M	F	M	F	M
	Mean ±SD		Mean ±SD		Mean ±SD		Mean ±SD	
2018	25	36	7.9 ± 0.7	6.3 ± 0.8	38.7 ± 1.9	36.8 ± 2.2	24.2 ± 2.0	19.7 ± 1.0
2019	14	17	7.7 ± 0.8	6.2 ± 0.8	39.2 ± 2.7	36.9 ± 1.9	24.9 ± 2.0	19.4 ± 0.9
2021	39	31	8.2 ± 0.9	6.7 ± 0.5	40.6 ± 2.2	38.3 ± 2.1	26.1 ± 2.4	20.4 ± 1.1
2022	44	23	8.0 ± 1.7	6.6 ± 0.4	40.2 ± 2.0	38.7 ± 1.7	25.0 ± 2.3	19.8 ± 1.8
Mean	122	107	8.0 ± 0.9	6.8 ± 1.6	39.9 ± 2.2	37.8 ± 6.5	25.2 ± 2.3	21.9 ± 4.8

Table 4. Results of two-way ANOVA testing for the effects of gender (male vs. female) and study year on *B. bombina* weight, length, and width measurements.

Measurement	Factor	df	F	p
Length (SVL)	Gender	1	45.83	< 0.001
	Year	3	10.62	< 0.001
	Gender × Year	3	0.45	0.72
	Error	221		
Width (SW)	Gender	1	403.47	< 0.001
	Year	3	6.08	< 0.001
	Gender × Year	3	1.25	0.29
	Error	221		
Weight	Gender	1	187.02	< 0.001
	Year	3	2.61	0.05
	Gender × Year	3	0.83	0.48
	Error	221		

Table 5. Separated weight groups of six-month-old *B. bombina* juveniles and their gender distribution within the resulting groups.

Group	n	Weigh mean ± SD	Male number (%)	Female number (%)
I	75	3.8 ± 0.3	4 (5.3 %)	71 (94.7 %)
II	75	3.1 ± 0.2	32 (42.7 %)	43 (57.3 %)
III	79	2.5 ± 0.3	71 (89.9 %)	8 (10.1 %)

its best chance for survival, and this is a way to save this locally endangered species from extinction.

During this study, we considered biosecurity and took all necessary precautions to reduce the risk of accidentally introducing pathogens along with the released *B. bombina*. A separate laboratory was built for *B. bombina* rearing to eliminate the risk of accidental contamination or transport of pathogens from other laboratories. Lab coats were required for working in the laboratory, and only people who worked on this project were allowed to enter the laboratory. Everyone who worked in the laboratory was familiar with the laboratory and biosafety protocols. Separate gloves, aquariums and cleaning equipment were used for each tank in order to avoid any possible contamination of pathogens from other tanks. For tank and laboratory disinfection, a saturated solution of table salt was used because *B. bombina* individuals are very sensitive to disinfectants and cleaning agents. Also, individuals were removed from the tanks during cleaning and disinfection. After disinfection, the tanks and laboratory were carefully washed with water in order to avoid leaving any residues of the disinfectant. Water in the tanks of *B. bombina* juveniles was changed daily because it gets contaminated quickly with leftover food and animal excreta. Tanks were filled only with fresh clean room temperature water.

In total, 229 tadpoles developed from the collected spawn during the study period. The percentage of *B. bombina* individuals which survived after metamorphosis (93.2%) and the overall survival of individuals (92.4%) during this study were very high (Table 2). The observed survival rate of tadpoles in natural habitats is only 6%. The survival percentage in this study was higher than 88% in all years except 2018. That year the tadpoles were grown under different conditions. The tadpoles were transferred to 120-liter aquariums in which the water level depth was about 25–30 cm. The aquariums were filled with live aquatic plants and 5–7 mm sized river pebbles, and water filters produced an effect of running water. The tadpoles began to die a month after they were transferred to these aquariums. The tadpoles were transferred from these unsuitable conditions to 60-liter plastic containers with live aquatic plants and river pebbles, in which water was standing still and its level was maintained at about 30 cm. After the growing conditions were changed the tadpoles stopped dying. During the subsequent years, the survival percentage of the tadpoles increased from 91.8% to 100% (Table 2). These results show that our egg incubation, rearing, and feeding methodology is effective and can be used for rearing this species in artificial conditions and releasing the adults into their natural habitats.

The survival results of *B. bombina* breeding under the laboratory conditions reported by other authors are consistent with our findings. Earlier studies showed mortality rates of tadpoles from 4 to 7% and juveniles at 8% (Kinne et al. 2006). In this study, the overall tadpole mortality was 6%, while for juveniles, mortality was

only 1%. As expected, the survival rate under artificial conditions was much higher than natural conditions because the factors important for the successful development and survival of *B. bombina* were kept stable, such as air and water temperature, water level, and the amount of food. Moreover, the individuals experienced only intraspecific competition for food or habitat, and they did not experience interspecific competition since they were grown without any other species present. There were no predators or species with which they needed to compete for food. In natural habitats, such interspecific competition is unavoidable, and the living conditions are not very stable. Furthermore, the artificially reared *B. bombina* individuals were repeatedly grouped by their size and kept in rather small numbers to reduce the intraspecific competition for food and preferred habitat.

In nature, *B. bombina* individuals reach sexual maturity at 2–4 years (Bülbül et al. 2018). Mature individuals can reach a snout-vent length of 56 mm (Lang 1988), but usually it ranges from 40 to 55 mm (Rimšaitė 2021). The *B. bombina* individuals reached maturity much earlier in the artificial conditions of this study than in their natural habitats. Our findings revealed that *B. bombina* individuals reached maturity at 8–9 months, when the males began vocalizing and some females spawned. The mean snout-vent lengths were 39.9 ± 2.2 mm for females and 37.8 ± 6.5 mm for males (Table 3). By comparison, the observed mean SVL of adult female *B. bombina* individuals was lower in some Romanian populations, e.g., 35.2 mm (Cogalniceanu and Miaud 2004) and 36.8 mm (Cogalniceanu and Miaud 2003). However, it was higher (47.1 mm) in Polish populations (Rafinska 1991). The mean SVL of male *B. bombina* individuals from the Romanian populations was also lower compared to our results, at 34.4 mm (Cogalniceanu and Miaud 2004) and 36.6 mm (Cogalniceanu and Miaud 2003).

Sexual size dimorphism (SSD) is present in over 90% of amphibian species (Nali et al. 2014), although it is weakly expressed in *B. bombina* (Cogalniceanu and Miaud 2004; Bülbül et al. 2018). Males have nuptial pads, which appear only in sexually mature individuals before the breeding season (Bülbül et al. 2018). However, we found a significant difference in measured SVL and SW between *B. bombina* males and females (Table 3). The weight of *B. bombina* juveniles also showed significant differences between the sexes. Female individuals were heavier than males. In contrast to our results, Cogalniceanu and Miaud (2004) in Romania and Bülbül et al. (2018) in Turkey found that adult *B. bombina* individuals in natural habitats did not show any significant differences in body mass or SVL between the sexes. A significant correlation was found between SVL and the age of a *B. bombina* population in Romania (Cogalniceanu and Miaud 2003).

The sex of amphibians is determined genetically, and many studies have shown that high temperatures result in male-biased populations, while female-biased populations develop in low temperatures (Cogalniceanu

and Miaud 2003). The number of males per pond is usually less than 10 in the northern distribution countries such as Latvia and Lithuania (Kuzmin et al. 2008). An unbalanced sex ratio affects the size of the population. Our rearing methods allowed us to predict the sex of six-month-old juveniles and individuals could be distributed into groups by their sex before they were mature. The multiyear threshold of *B. bombina* juvenile weight may be used for sex determination with >90% accuracy. This is very useful because this method enables the release of individuals into natural ponds with an equal sex ratio even before they are mature. Such knowledge provides a valuable tool for the management of a declining population by balancing the unbalanced sex ratio in naturally threatened *B. bombina* populations.

Overall, the conservation methods presented here can allow us to save small vulnerable populations of *B. bombina* and prevent them from extinction by maintaining their genetic diversity and releasing individuals into more favorable habitats that are usually unavailable due to their very segregated distribution. Such efforts will help preserve the declining populations of the species at the northern edge of their range for future generations and prevent this species from complete extinction where their habitats are much too fragmented.

Acknowledgments

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Literature Cited

Berzins A. 2003. The Fire-bellied Toad *Bombina bombina* Linnaeus, 1761. Pp. 82–83 In: *Red Book of Latvia*. Editor, Andrušaitis G. LU Biological Faculty, Riga, Latvia. 691 p.

Bülbül U, Eroğlu Aİ, Kurnaz M, Kaya ZM, Koç H, Eroğlu SA. 2018. Age structure and some growth parameters in a population of *Bombina bombina* (L., 1761) from Turkey. *Russian Journal of Herpetology* 25(3): 221–226.

Čeirans A, Pupina A, Pupins M. 2020. A new method for the estimation of minimum adult frog density from a large-scale audial survey. *Scientific Reports* 10(1): 1–12.

Chikhlyayev IV, Ruchin AB. 2021. An overview of helminths of the European Fire-bellied Toad *Bombina bombina* (Amphibia, Anura) in the Volga River Basin. *Biosystems Diversity* 29(4): 407–414.

Cogalniceanu D, Miaud C. 2003. Population age structure and growth in four syntopic amphibian species inhabiting a large river floodplain. *Canadian Journal of Zoology* 81(6): 1,096–1,106.

Cogalniceanu D, Miaud C. 2004. Variation in life history traits in *Bombina bombina* from the lower Danube floodplain. *Amphibia-Reptilia* 25(1): 115–119.

Fedorowicz Z. 1918. Materyały do herpetologii Litwy i Rusi Białej. *Pamiętnik Fizyograficzny* 25: 1–12.

Frankham R, Ralls K. 1998. Conservation biology: inbreeding leads to extinction. *Nature* 392: 441–442.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.

IUCN. 2023. The IUCN Red List of Threatened Species. Version 2022-2. Available: <https://www.iucnredlist.org> [Accessed: 2 May 2023].

Ivinskis P, Rimšaitė J. 2011. Nauji duomenys apie 2010 m. stebėtos saugomos gyvūnų rūšys. *Raudoni Lapai* 13: 43–44.

Kinne O, Kunert J, Zimmermann W. 2006. Breeding, rearing, and raising the Red-bellied Toad *Bombina bombina* in the laboratory. *Endangered Species Research* 1: 11–23.

Kurnaz M, Kutrup B. 2019. New distribution data of the vulnerable *Mertensiella caucasica* from Gümüşhane, Turkey. *Nature Conservation Research* 4(1): 109–111.

Kuzmin SL, Pupina A, Pupins M, Trakimas G. 2008. The northern border of the distribution of the Red-bellied Toad (*Bombina bombina*). *Zeitschrift für Feldherpetologie* 15(2): 215–228.

Lang M. 1988. Notes on the genus *Bombina* Oken (Anura, Bombinatoridae). *The British Herpetological Society Bulletin* 26: 3–13.

Michaels CJ, Antwis RE, Preziosi RF. 2015. Impacts of UVB provision and dietary calcium content on serum vitamin D₃, growth rates, skeletal structure, and coloration in captive Oriental Fire-bellied Toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition* 99(2): 391–403.

Nali RC, Zamudio KR, Haddad CF, Prado CP. 2014. Size-dependent selective mechanisms on males and females and the evolution of sexual size dimorphism in frogs. *The American Naturalist* 184(6): 727–740.

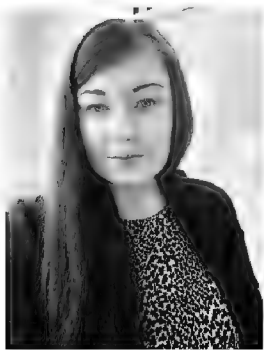
Nicoara A, Nicoara M. 2007. Study of a *Bombina bombina* (Anura, Amphibia) population from the periurban ecosystems north of Iași city. *Herpetologica Romanica* 1: 22–29.

Pupina A, Pupins M, Nekrasova O, Tytar V, Kozynenko I, Marushchak O. 2018. Species distribution modelling: *Bombina bombina* (Linnaeus, 1761) and its important invasive threat *Perccottus glenii* (Dybowski, 1877) in Latvia under global climate change. *Environmental Research, Engineering & Management* 74(4): 79–86.

Pupina A, Pupins M. 2007. Sarkanvēdera ugunskrupis *Bombina bombina* (Linnaeus, 1761) un tā aizsardzība Latvijā. *Latvian Ecological Biedrība* 2007: 1–143.

Pupina A, Pupins M. 2008. The new data on distribution, biotopes, and situation of populations of *Bombina bombina* in the south-eastern part of Latvia. *Acta Biologica Universitatis Daugavpiliensis* 8(1): 67–73.

- Pupina A, Pupins M. 2009. Comparative analysis of biotopes and reproductive-ecological manifestations of *Bombina bombina* (Linnaeus, 1761) in Latvia. *Acta Biologica Universitatis Daugavpiliensis* 9(1): 121–130.
- Pupina A, Pupins M. 2012. Invasive fish *Perccottus glenii* in biotopes of *Bombina bombina* in Latvia on the north edge of the Fire-bellied Toad's distribution. *Acta Biologica Universitatis Daugavpiliensis* 12: 82–90.
- Pupina A, Pupins M. 2016. Action plan for the Fire-bellied Toad *Bombina bombina* in Latvia: assessment of the implementation for ten years, releasing from aquaculture and restoration of habitats in 2006–2016. *Acta Biologica Universitatis Daugavpiliensis* 16(2): 201–211.
- Pupina A. 2011. Sarkanvēdera ugunskrupju *Bombina bombina* L. ekoloģijas īpatnības uz sugas areāla ziemeļu robežas Latvijā. Ph.D. Dissertation, Daugavpils Universitāte, Daugavpils, Latvia.
- Rafinska A. 1991. Reproductive biology of the Fire-bellied Toads, *Bombina bombina* and *B. variegata* (Anura: Discoglossidae): egg size, clutch size, and larval period length differences. *Biological Journal of the Linnean Society* 43(3): 197–210.
- Rimšaitė J. 2021. Radonpilvė kūmutė. *Bombina bombina* (Linnaeus, 1758). Pp. 207 In: *Red Data Book of Lithuania. Animals, Plants, Fungi*. Editor, Rašomavičius V. Lututė, Vilnius, Latvia. 684 p.
- Schröder C, Pokorný I, Dolgener N, Herden C, Drews H, Tiedemann R. 2012. Allochthonous individuals in managed populations of the Fire-bellied Toad, *Bombina bombina*: genetic detection and conservation implications. *Limnologica* 42(4): 291–298.
- Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet PA, Crnobrnja-Isailović J, Denoël M, Ficetola GF, Gonçalves J, et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia* 35: 1–31.
- SRIS. 2023. Saugomų rūšių informacinė sistema (Information system of protected species). Available: <https://sris.am.lt/> [Accessed: 2 May 2023].
- Stuart S, Hoffmann M, Chanson J, Cox N, Berridge R, Ramani P, Young B, Editors. 2008. *Threatened Amphibians of the World*. Lynx Edicions, Barcelona, Spain; IUCN, Gland, Switzerland; and Conservation International, Arlington, Virginia, USA. 134 p.
- Tytar V, Nekrasova O, Pupina A, Pupins M, Oskyrko O. 2018. Long-term bioclimatic modeling of the distribution of the Fire-bellied Toad, *Bombina bombina* (Anura, Bombinatoridae), under the influence of global climate change. *Vestnik Zoologii* 52(4): 341–348.
- Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11,466–11,473.
- Wojdan D. 2010. Impact of vehicle traffic on amphibian migrations in the protection zone of the Swietokrzyski National Park. *Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego* 7: 466–472.



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A new species of Sipo Snake, *Chironius* (Serpentes: Colubridae), from the Yungas of Bolivia

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Abstract.—A new snake of the genus *Chironius* is described based on external morphological characters and phylogenetic evidence. The new species occurs in Bolivia, both in the humid montane forests of the Yungas of Cochabamba and in Santa Cruz. It differs from all congeners in having 10 dorsal scale rows at midbody, an entire cloacal plate, keeled paravertebral rows, lightly colored lower portions of the supralabials, a yellow snout, a short hemipenis, and lacking postocular stripes, proximal enlarged spines on the hemipenis, and apical pits. Adults and juveniles have an emerald green background color. The new species is recovered as the sister taxon of *C. leucometapus*, which is known from the Amazonian slopes of the Andes between central Peru and northern Ecuador. We also provide an identification key to the species of *Chironius* with 10 dorsal rows at midbody.

Keywords. Carrasco National Park, hemipenes, phylogeny, reptiles, Squamata, systematics

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Introduction

The genus *Chironius* Fitzinger, 1826 currently contains 24 terrestrial or semi-arboreal species, five of which have been described in the last 15 years (Entiauspe-Neto et al. 2020; Fernandes and Hamdan 2014; Hamdan and Fernandes 2015; Kok 2010; Sudré et al. 2024). Commonly known as Sipo Snakes, they are unique among Neotropical snakes in having 10 or 12 longitudinal rows of dorsal scales around the midbody (Dixon et al. 1993; Hollis 2006). Species of *Chironius* occur from Honduras in Central America to Uruguay and Argentina in South America (Dixon et al. 1993). The monophyly of *Chironius* is strongly supported by both morphological and DNA-sequence data (Hamdan et al. 2017; Hollis 2006; Klaczko et al. 2014; Torres-Carvajal et al. 2019b).

Following the comprehensive taxonomic revision of *Chironius* by Dixon et al. (1993), along with subsequent

taxonomic work, nine species of *Chironius* are currently recognized in Bolivia (Embert 2007; Wallach et al. 2014): *C. exoletus* Linnaeus, 1758, *C. flavolineatus* Jan, 1863, *C. fuscus* Linnaeus, 1758, *C. laurenti* Dixon, Wiest, and Cei, 1993, *C. maculovenstris* Dixon, Wiest, and Cei, 1993, *C. monticola* Roze, 1952, *C. multivenstris* Schmidt and Walker, 1943, *C. quadricarinatus* Boie, 1827, and *C. scurrula* Wagler, 1824. Recent herpetological field work in the Bolivian-Peruvian Yungas of Carrasco National Park (Cochabamba, Bolivia) and the Tucuman-Bolivian Yungas of Santa Cruz yielded several specimens of *Chironius* that could not be assigned to any of the described species. Herein we describe them as a new species based on morphological and phylogenetic evidence.

Materials and Methods

Specimen Sampling and Morphological Data

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In addition to the type series listed below, specimens examined in this study are listed in Appendix 1. Specimens were collected under permits MMAyA-VMA-DGBAP-004 and VMABCCGDF N° 16/19 issued by Ministerio de Medio Ambiente y Aguas and sacrificed in the field by intracardiac injection of 1% xylocaine solution. Immediately after euthanasia, muscle tissue samples were extracted from all specimens and preserved in 96% ethanol. Specimens were subsequently fixed in 10% formalin, stored in 75% ethanol, and deposited in the Bolivian herpetological collections of the Museo de Historia Natural Alcide d'Orbigny (MHNC-R), Cochabamba, and Museo de Historia Natural Noel Kempff Mercado (MNKR), Universidad Autónoma Gabriel René Moreno, Santa Cruz de La Sierra.

We followed the terminology proposed by Dowling (1951) and Dixon et al. (1993) for scale counts and measurements. Snout-vent length and tail length were recorded using a measuring tape to the nearest 0.1 mm. All other measurements were made with digital calipers to the nearest 0.01 mm (rounded to the nearest 0.1 mm) and included: head width (HW), at widest point of head between mouth commissures; head length (HL), from tip of rostral to posterior border of last supralabial; horizontal eye diameter (EL); eye-nostril distance (EN), from posterior border of nostril to anterior corner of eye; snout length (SL), from rostral tip to anterior margin of eye; maximum loreal length; and maximum loreal height. The morphological and hemipenial terminology follows Dowling and Savage (1960) and Dixon et al. (1993). Color was recorded for both living and preserved specimens. Maxillary teeth were counted *in situ* on the right side. Sex was determined by searching for hemipenes either by eversion or through a ventral incision at the base of the tail. We prepared the left hemipenis of paratype MHNC-R 3229 (SVL = 810 mm) following standard techniques (Pesantes 1994). We compared data from the specimens examined with literature data from Dixon et al. (1993) and Torres-Carvajal et al. (2019a).

DNA Data and Phylogenetic Analyses

We obtained new DNA sequences from the holotype of the new species described here for three mitochondrial genes, the small (*12S*, 424 aligned bp) and large (*16S*, 444 aligned bp) ribosomal subunit genes, and subunit IV of NADH dehydrogenase (*ND4*, 693 aligned bp), as well as one nuclear gene, the oocyte maturation factor *mos* (*c-mos*, 537 aligned bp). The muscle tissue sample was mixed with Proteinase K and lysis buffer and digested overnight. Total genomic DNA was extracted using a guanidinium isothiocyanate extraction protocol. DNA samples were quantified using a Nanodrop® ND-1000 (NanoDrop Technologies, Inc.), re-suspended, and diluted to 25 ng/μl in ddH₂O prior to amplification. Primers and amplification protocols follow Torres-Carvajal et al. (2019b), and we added the new sequences

to the dataset in that study to produce a matrix of 173 taxa and 2,098 aligned nucleotides. GenBank accession numbers of the sequences produced in this study are PP408265 (*12S*), PP408266 (*16S*), PP411803 (*ND4*), and PP411804 (*c-mos*).

Data were assembled and aligned in Geneious Prime 2022.1.1 (<https://www.geneious.com>) under default settings for MUSCLE 3.8.425 (Edgar 2004). Protein-coding sequences were translated into amino acids for confirming the alignment and absence of pseudogenes. After partitioning the concatenated dataset by gene, we ran a maximum likelihood analysis in RAxML v8.2.10 (Stamatakis 2014) under the GTRGAMMA model for each partition. We assessed nodal support with the rapid bootstrapping (BS) algorithm (Stamatakis et al. 2008) on 1,000 replicates. We executed these analyses in the CIPRES Science Gateway (Miller et al. 2010). Outgroup taxa were the same as in Torres-Carvajal et al. (2019b). We used DIVEIN (Deng et al. 2010) to calculate *16S* uncorrected pairwise genetic distances between the new species described herein and other species of *Chironius*, and compared those distances with recently published data (Sudré et al. 2024).

Species Concept

In this work we follow the unified species concept (de Queiroz 1998, 2007). We infer the existence of the new species described below based on morphological and phylogenetic criteria, which we interpret as evidence of lineage separation.

Results

Systematic Account

Chironius whipala sp. nov.

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Proposed common English names: Whipala Sipos, Whipala snakes

Proposed common Spanish names: Sipos Whipala, serpientes Whipala

Holotype. MHNC-R 3099 (Fig. 1), an adult male collected in Chaquisacha, Carrasco National Park, Cochabamba Department, Bolivia (17°24'24.42"S, 65°15'34.88"W, 1,337 m) on 21 February 2021 by Rene Carpio-Real at 2345 h in humid montane forests (Yungas).

Paratypes (12). *Cochabamba Department:* Same collection data as holotype: MHNC-R 3100, juvenile male collected by Oliver Quinteros-Muñoz on 15 March 2021 at 2330 h; MHNC-R 3101, adult female collected by Rene Carpio-Real on 17 December 2020 at 2300 h; MHNC-R 3102, adult male (specimen in poor condition,

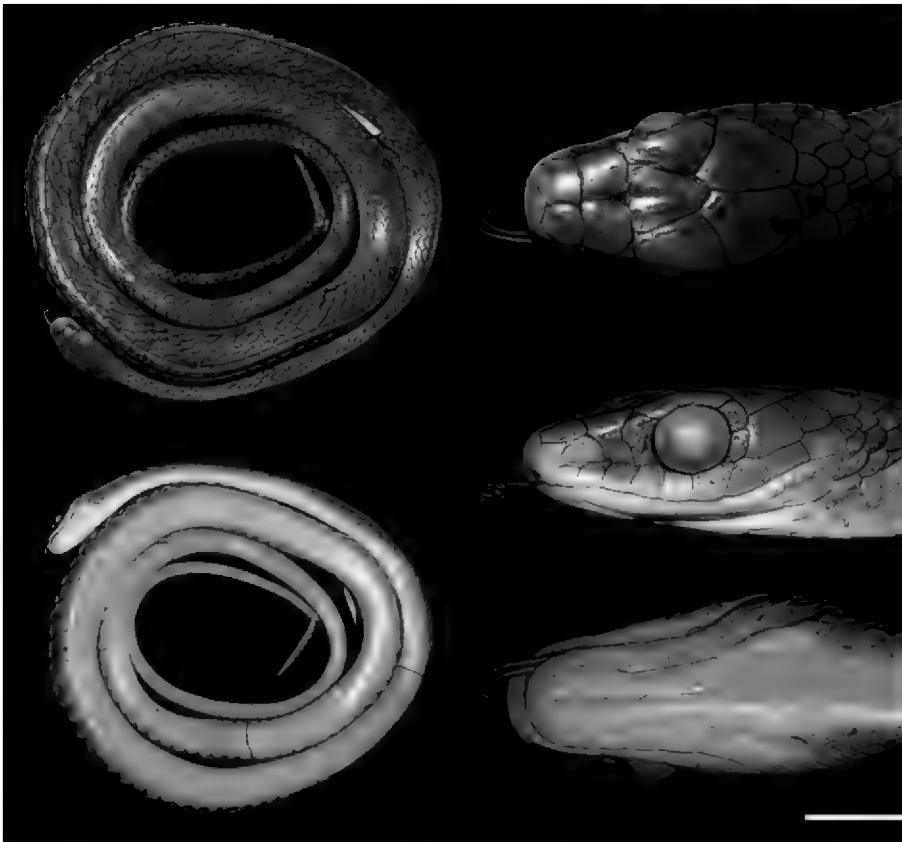


Fig. 1. Male holotype (MHNC-R 3099) of *Chironius whipala* sp. nov. in preservative. Left: dorsal (top) and ventral (bottom) views of body. Right: dorsal (top), lateral (middle), and ventral (bottom) views of head. Scale bar (head only) = 10 mm. Photographs by Rene Carpio-Real.

found dead), collected by José Balderrama on 27 September 2020; MHNC-R 3133, adult male collected by Oliver Quinteros-Muñoz on 17 April 2021 at 1930 h; MHNC-R 3134, juvenile female collected by Rene Carpio-Real on 20 April 2021 at 2349 h; MHNC-R 3135 juvenile female collected by Rene Carpio-Real on 17 May 2021 at 2152 h; MHNC-R 3227, an adult male found dead in poor condition, collected by Jorge Espinoza on 17 December 2022 at 1500 h near La Pajcha close to type locality, 2,000 m; MHNC-R 3229, an adult male found dead, collected by Bladimir Marca on 25 May 2023 at 1146 h near Arepuchco close to type locality, 1,270 m. *Santa Cruz Department*: MNKR 3073, adult male collected by Walter Romero on 28 October 2001 in Pampagrande, Florida Province, 18°6'0.02"S, 64°6'0.00"W, 1,300 m; MNKR 3589, adult male collected by Pedro Maida on 18 December 2003 in La Hoyada, Florida Province, 17°55'12.02"S, 64°7'12.01"W, 1,730 m; MNKR 4833 adult male and MNKR 4834 adult female, collected by Lucindo Gonzales, Rutty Rodriguez, and Oswaldo Helmig on 15 November 2009 in Laja Tocos, Vallegrande Province, 18°29'41.37"S, 63°43'55.34"W, 1,300 m.

Etymology. The specific name “*whipala*” comes from the original Aymara language, which means “emblem,” i.e., the emblem of the original people of the Andes of Bolivia and an emblem that honors and symbolizes respect for our Pachamama (Mother Earth). According to an anonymous Aymara phrase, “where there is a wiphala, love and respect for Mother Earth (Pachamama) and the universe will be represented.”

Diagnosis. *Chironius whipala* can be distinguished from other species of *Chironius* by the following combination

of characters: (1) dorsal scale formula 10-10-8 in males, 10-10-10 in females; (2) apical pits absent; (3) paravertebral keels present, inconspicuous in females; (4) ventrals 149–151 in males, 151–155 in females; (5) subcaudals 117–122 in males, 119–120 in females; (6) cloacal plate single; (7) loreal slightly longer than high; (8) maxillary teeth 32; (9) juveniles emerald green or olive green, without markings; (10) adults emerald green, unmarked; (11) black postocular stripe absent; (12) snout yellow; (13) ventrals and subcaudals yellowish or greenish, immaculate; (14) hemipenis short (i.e., ~2X as long as wide), cylindrical, unilobed, with undivided *sulcus spermaticus* and base covered with tiny spines.

Chironius whipala differs from all known species of *Chironius* except *C. fuscus* and *C. leucometapus* in having 10 dorsal scale rows at midbody, an entire cloacal plate, lightly colored lower portion of supralabials, and keeled paravertebral rows. From *C. fuscus* (character states in parentheses), *C. whipala* can be distinguished by having a yellow snout and forehead (head uniformly colored) and lacking both a postocular stripe (present) and enlarged spines on the proximal aspect of the hemipenial body (large spines present) (Dixon et al. 1993; Torres-Carvajal et al. 2019a). From *C. leucometapus* (character states in parentheses), *C. whipala* can be distinguished by having a yellow snout patch covering rostral, first supralabials, and anterior portion of nasals and internasals (rusty brown or coppery orange snout patch covering rostral, first pair of supralabials, internasals, prefrontals, nasals, frontal, and anterior half of supraoculars). Finally, *C. whipala* differs from both *C. fuscus* and *C. leucometapus* in having a much shorter hemipenis (~2X versus > 5X as long as wide) and in lacking apical pits on the dorsal scales.

Description of holotype. Adult male (Fig. 1), total length (TTL) 1,356 mm, SVL 960 mm, tail incomplete; head well differentiated from neck, narrow anteriorly, wider in temporal region, HW 43.6% of HL; snout rounded in dorsal and lateral views, SL 11.4 mm; eye large (EL 6.5 mm), pupil round, EN 20.8% of HL; rostral large, wider than high, visible from above; nasal divided, with large nostril separating anterior half from posterior half; internasals quadrangular, as wide as long, smaller than prefrontals, laterally in contact with nasals; prefrontals slightly wider than long, larger than internasals, each laterally in contact with nasal, loreal, and preocular; frontal bell-shaped, longer than wide, twice the length of suture between prefrontals; parietals large, 1.4 times as long as wide, interparietal suture length similar to length of frontal; postcephalic scales four; preocular single, separated from frontal by prefrontal-supraocular contact; loreal slightly longer than high, in contact with nasal, prefrontal, preocular, and supralabials II and III; postoculars two on each side; temporals 1 + 1; supralabials nine, I in contact with nasal, II in contact with nasal and loreal, III in contact with loreal and

preocular, IV - VI in contact with orbit (VI also in contact with postocular), VII and VIII in contact with anterior temporal, and IX in contact with both anterior and posterior temporals; infralabials 10/9, 6/5 in contact with chinshields; chinshields in two pairs, anterior pair shorter than posterior pair, both pairs in contact medially, except for last half of posterior pair; maxillary teeth 32; dorsal scales in 10-10-8 rows, smooth without apical pits; ventrals 151; cloacal plate single.

Color of holotype in life. Dorsal background uniformly emerald green, with grey skin between scales; snout yellow; lower portion of supralabials yellowish cream; ventral aspect of head immaculate white; dorsal coloration extending onto lateral tips of ventrals; ventral coloration white anteriorly, yellowish green on first third of body, turning into green tones posteriorly.

Color of holotype in preservative. Dorsum uniformly bluish-green, darker on posterior two-thirds of body; dorsal aspect of head dark brown; snout light brown; lower portion of supralabials and ventral aspect of head cream; venter bluish grey, lighter anteriorly (Fig. 1).

Hemipenis. The hemipenial description is based on the left organ of MHNC-R 3229, an adult male with SVL = 810 mm (Fig. 2). Organ relatively short, ~2X as long as wide, unilobed, non-capitate, and subcylindrical in shape; *sulcus spermaticus* undivided, linear, running centripetally from base to apex and terminating near center of apex, bordered by naked tissue proximally, spines centrally, and papillae distally; base of organ covered with small spines; basal naked pocket on medial region; proximal half of body densely covered with large spines; distal half of body densely covered with small spines (less than half the size of proximal spines) proximally, which are replaced by papillate fringes and calyces with papillate borders distally; apex with nude area.

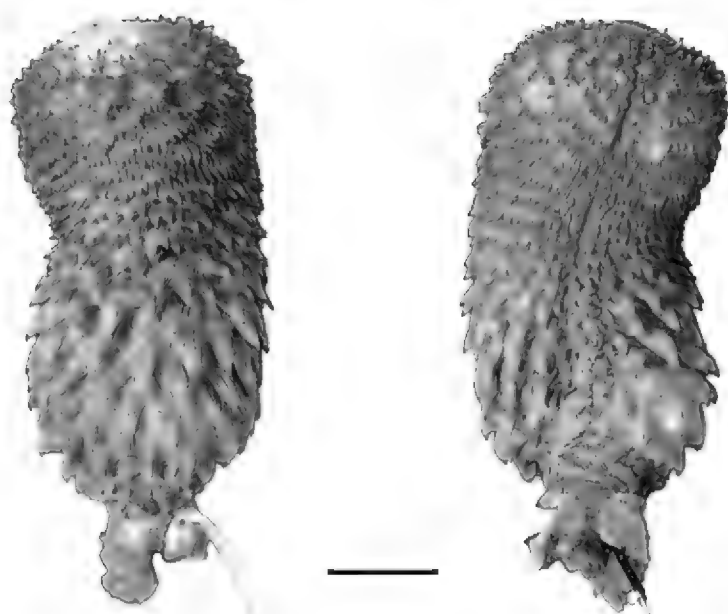


Fig. 2. Left hemipenis of *Chironius whipala* sp. nov. (MHNC-R 3229) in asulcate (left) and sulcate (right) views. Scale bar = 5 mm. Photographs by Diego A. Paucar.

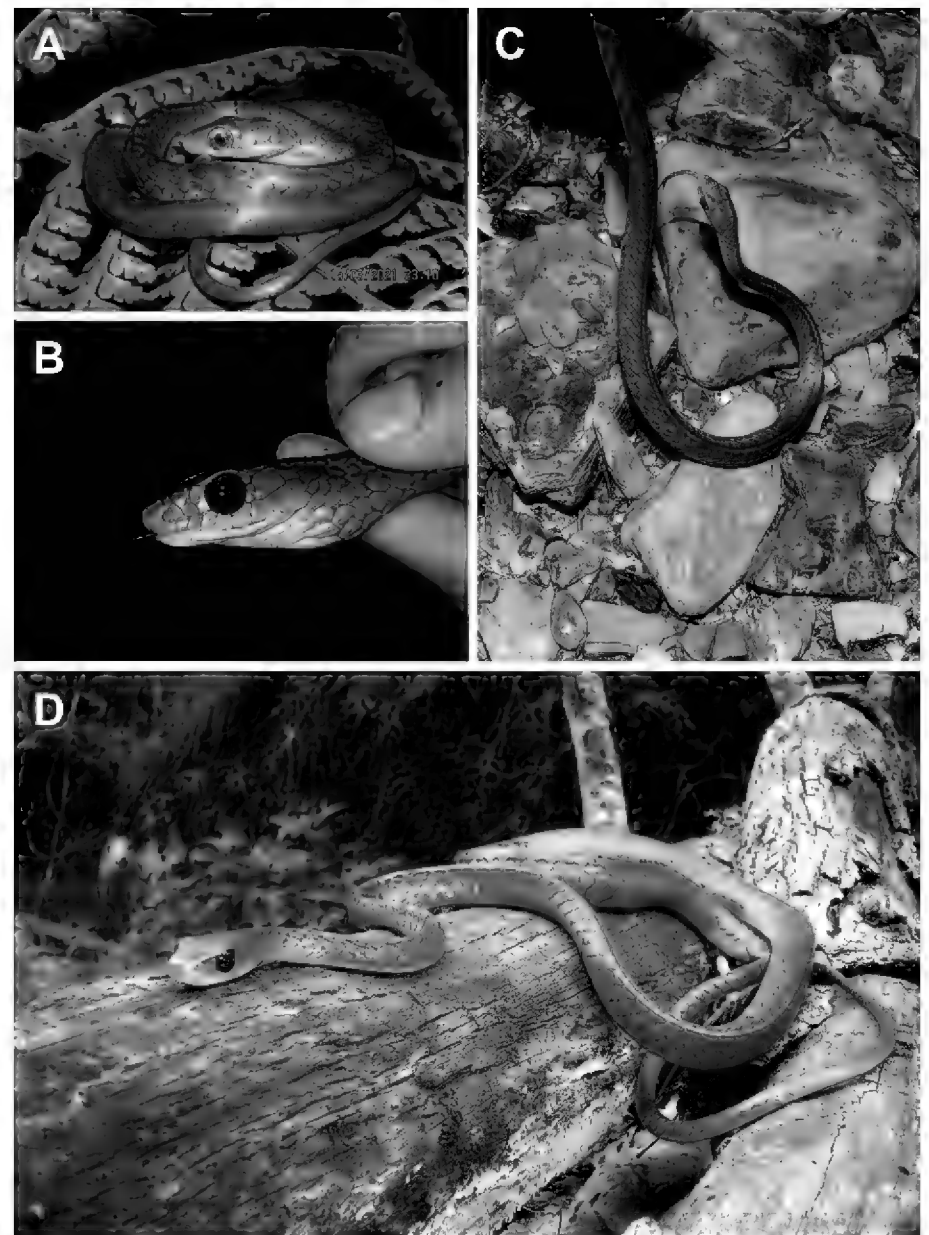


Fig. 3. General view of *Chironius whipala* sp. nov. in life. (A) juvenile male, paratype MHNC-R 3100; (B) adult male, paratype MHNC-R 3133; (C) juvenile female, paratype MHNC-R 3135; (D) adult female, paratype MNKR 4834. Photographs by Oliver Quinteros-Muñoz (A, B, C) and Lucindo Gonzales (D).

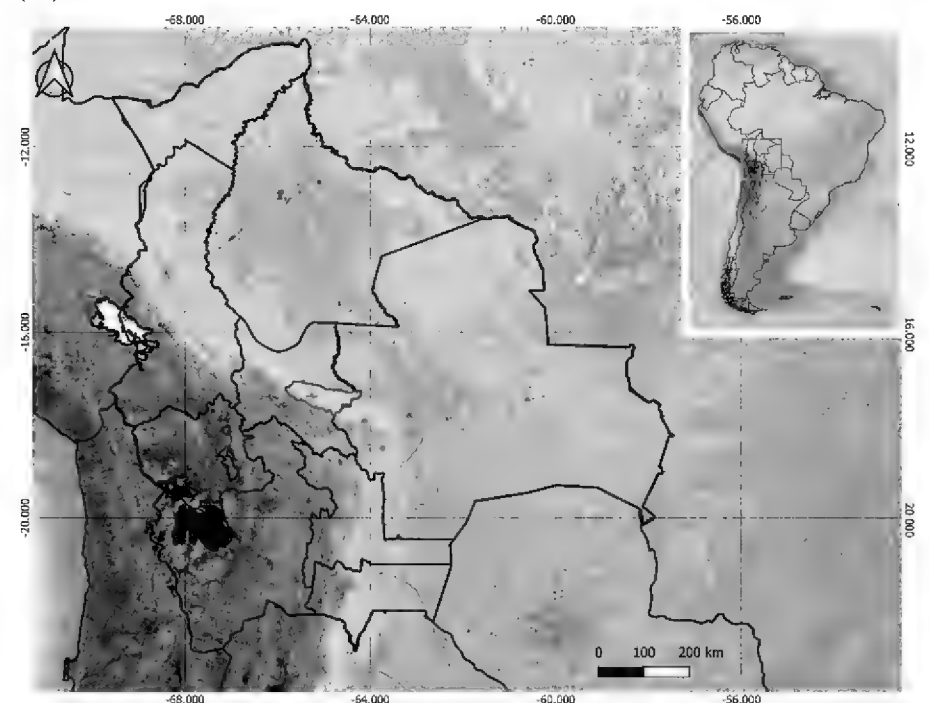


Fig. 4. Known distribution of *Chironius whipala* sp. nov. in Bolivia. The star corresponds to the type locality, and the green line depicts the boundaries of Carrasco National Park.

Variation. Intraspecific variations of *Chironius whipala* sp. nov. in scutellation and meristic characters are presented in Table 1. While males present reduction in the number of dorsal scales (10-10-8), females maintain the same number along the body (10-10-10). All specimens are similar in color pattern (Fig. 3). Female juvenile MHNC-R 3134 (SVL 260 mm) and male juvenile MHNC-R 3100 (SVL 277 mm) have faint, light transverse bands along body, which are separated from

Table 1. Sexual variation in selected meristic and morphometric characters for *Chironius whipala* **sp. nov.** Range (first line) and mean \pm SD (second line) are presented when appropriate (n = sample size). Abbreviations are defined in the Materials and Methods section.

Character	Males (n = 9)	Females (n = 4)
Dorsal scale formula	10–10–8	10–10–10
Ventrals	149–156 151.11 \pm 2.09	151–155 153.25 \pm 1.71
Subcaudals	114–122 118.43 \pm 3.05	119–120 119.67 \pm 0.58
Supralabials	8–10, usually 9	8–9, usually 9
Supralabials entering orbit	4–5–6 (n = 8) 5–6–7 (n = 1)	4–5–6
Infralabials	8–10, usually 9	8–10, usually 9
SL/HL	0.36–0.41 0.39 \pm 0.02	0.37–0.39 0.38 \pm 0.01
EL/EN	1.00–1.5 1.19 \pm 0.16	1.07–1.37 1.28 \pm 0.15
HW/HL	0.37–0.67 0.50 \pm 0.11	0.39–0.56 0.45 \pm 0.08
Loreal height/length	0.62–0.82 0.70 \pm 6.53	0.78–0.94 0.85 \pm 6.83
Maximum SVL in mm	1,082	912
TL/SVL in adults	0.47–0.54 0.49 \pm 0.02	0.51



Fig. 5. General habitat of *Chironius whipala* **sp. nov.**, near Chaquisacha, Carrasco National Park, Cochabamba, Bolivia. Photograph by Oliver Quinteros-Muñoz.

each other by ~3 scales.

Distribution and ecology. *Chironius whipala* **sp. nov.** is known from the type locality and immediate surroundings in Carrasco National Park, Cochabamba Department, as well as from Santa Cruz Department (Figs. 4–5). This species occurs in the Bolivian-Peruvian Yungas and Tucuman-Bolivian Yungas ecoregions (Ibisch and Mérida 2003). All specimens were collected at night between 1,270 and 2,000 m asl. Most of them were found lying on the vegetation at heights from 1–6 m above ground during the rainy season. Stomach contents in one of the specimens (MHNC-R 3102, adult male) included remains (hind leg) of a specimen of *Oreobates* sp., one undigested adult specimen of *Pristimantis* cf. *reichlei*, and one undigested adult specimen of “*Cercosaura manicata boliviana*.” An adult female contained seven eggs (average length/width = 40.1 mm/15.6 mm.) Within its range, *C. whipala* **sp. nov.** is found in sympatry with three congeners: *C. exoletus*, *C. laurenti*, and *C. monticola*.

Phylogenetic relationships. The phylogenetic tree recovered in this study (Fig. 6) is generally congruent with the tree presented by Torres-Carvajal et al. (2019b). The new species is the sister taxon of *Chironius*

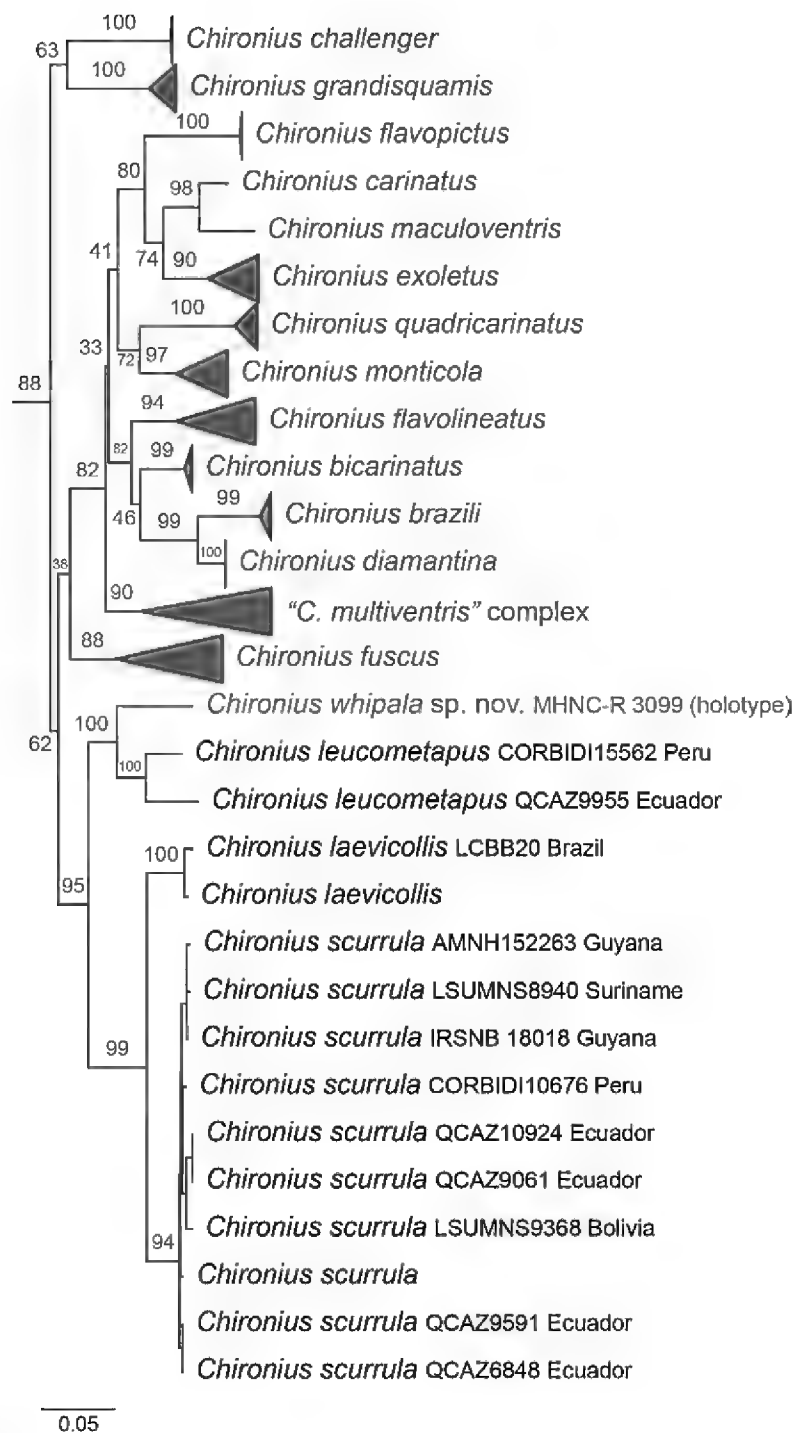


Fig. 6. Phylogeny of *Chironius*. Maximum likelihood tree obtained from a RAxML analysis of a concatenated matrix of 173 terminals and three mitochondrial (*12S*, *16S*, *ND4*) and one nuclear (*c-mos*) genes. Numbers along branches correspond to bootstrap support values. For most species, individual species clades are collapsed; outgroup taxa are not shown. For uncollapsed clades, species name followed by voucher number and country (if available) are provided. Except for the holotype of *C. whipala*, other details are available in Torres-Carvajal et al. (2019b).

leucometapus and, together with their sister clade (*C. laevis*, *C. scurrula*), they form a strongly supported clade (BS = 95). The latter clade is the sister taxon of all remaining species of *Chironius* except for sister taxa *C. challenger* and *C. grandisquamis*. Uncorrected pairwise

genetic distances (*16S*) between *C. whipala* sp. nov. and its closest relatives *C. leucometapus*, *C. laevis*, and *C. scurrula* are 3.4%, 6%, and 5.6%, respectively. The *16S* genetic distance between the two specimens of *C. leucometapus* included in this study is 1.9%.

Discussion

Like other animal taxa, the diversity and systematics of *Chironius* snakes from Bolivia have been poorly studied. For example, in a recent comprehensive phylogeny of *Chironius*, only two of the 97 samples were from Bolivia (Torres-Carvajal et al. 2019b), indicating that access to samples from Bolivia is limited. Here we describe a new species of *Chironius* endemic to the Bolivian Yungas (Bolivian-Peruvian and Tucuman-Bolivian) based on morphological and phylogenetic evidence. This adds to the recent descriptions of new species of sipo snakes from Brazil (Entiauspe-Neto et al. 2020; Sudré et al. 2024), increasing the number of *Chironius* species to 25 (including 10 in Bolivia).

Our phylogenetic analysis suggests that *C. whipala* sp. nov. is the sister species of *C. leucometapus* (Fig. 6), which is known from the eastern slopes of the Andes from central Peru to northern Ecuador (Torres-Carvajal et al. 2019a). The closest record of *C. leucometapus* (Rupa Rupa, Huánuco, Peru) lies about 1,428 km NW of the type locality of *C. whipala*. Although both species are similar at first glance in having a lightly colored snout (yellowish in *C. whipala* and rusty brown to coppery orange in *C. leucometapus*), they differ notably in the relative length of the hemipenis. Genetic distances represent another criterion supporting the recognition of the new species described in this paper. The uncorrected *16S* genetic distance between *C. whipala* and its sister species *C. leucometapus* (3.4%) is similar to those of several *Chironius* species pairs, such as *C. brazili* and *C. grandisquamis* (3.1–3.5%), or *C. diamantina* and *C. dracomaris* (3.1–3.7%); and within *Chironius*, *16S* distances range from 0.4% (*C. bicarinatus*/*C. gouveai*) to 10.5% (*C. flavolineatus*/*C. leucometapus*) (Sudré et al. 2024). Moreover, the *16S* distance between the two specimens of *C. leucometapus* included in this study,

Key to the species of *Chironius*

- 1. Cloacal plate single 2
 - Cloacal plate divided *C. grandisquamis*
- 2. At least lower portion of supralabials lightly colored 3
 - All supralabials black or dark colored *C. laevis*
- 3. Paravertebral scale rows keeled 4
 - Paravertebral scale rows not keeled *C. scurrula*
- 4. Snout and forehead yellow or rusty; no basal large spine on hemipenis 5
 - Head uniformly colored; basal large spine on hemipenis *C. fuscus*
- 5. Apical pits present; hemipenis ~5X as long as wide *C. leucometapus*
 - Apical pits absent; hemipenis ~2X as long as wide *C. whipala* sp. nov.

which were collected from two localities that lie ~930 km apart (Pastaza province in Ecuador and Huánuco department in Peru), is much lower (1.9%) than the distance between *C. whipala* and *C. leucometapus*, which further supports recognition of the new species described here.

Chironius whipala **sp. nov.** is endemic to the Bolivian humid montane forests known as the Bolivian-Peruvian Yungas (Figs. 4–5) in Carrasco National Park, Cochabamba, and the Tucuman-Bolivian Yungas, Santa Cruz. Recently, Gonzales et al. (2020) described *Oxyrhopus emberti*, another snake species endemic to the Bolivian Yungas, which suggests that the diversity of snakes within this region needs further study. The Yungas ecoregions include significant landscape heterogeneity and altitudinal variation, which probably have facilitated the impressive diversification of different groups of fauna and flora (Navarro and Maldonado 2004; Vásquez et al. 2003). The Yungas of central Bolivia, where *C. whipala* was first discovered, is the area with the greatest number of endemic species of amphibians (de la Riva and Reichle 2014). For these reasons, the Yungas ecoregion should be given priority in terms of the exploration and conservation of Bolivia's herpetofauna.

Key to Species of *Chironius* with 10 Rows of Dorsal Scales around the Midbody

The above key is a modification of the key presented by Dixon et al. (1993) and only includes the species with 10 longitudinal rows of dorsals around midbody. All other species of *Chironius* have 12 rows of dorsals and were excluded from this key because the corresponding and significant taxonomic work is beyond the scope of this paper.

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Literature Cited

- de la Riva I, Reichle S. 2014. Diversity and conservation of the amphibians of Bolivia. *Herpetological Monographs* 28: 46–65.
- Deng W, Maust BS, Nickle DC, Learn GH, Liu Y, Heath L, Kosakovsky Pond SL, Mullins JI. 2010. DIVEIN: a web server to analyze phylogenies, sequence divergence, diversity, and informative sites. *BioTechniques* 48: 405–408.
- de Queiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation. Pp. 57–75 In: *Endless Forms: Species and Speciation*. Howard DJ, Berlocher SH, Editors. Oxford University Press, Oxford, United Kingdom. 496 p.
- de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
- Dixon JR, Wiest JA, Cei JM. 1993. *Revision of the Neotropical Snake Genus Chironius Fitzinger (Serpentes, Colubridae)*. Monografie XIII. Museo Regionale di Scienze Naturali, Torino, Italy. 279 p.
- Dowling HG. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1,792–1,797.
- Embert D. 2007. Distribution, diversity, and conservation status of Bolivian reptiles. Doctoral Dissertation, Rheinischen Friedrichs-Wilhelms-Universität Bonn, Bonn, Germany.
- Entiauspe-Neto OM, Lyra ML, Koch C, Quintela FM, Abegg AD, Loebmann D. 2020. Taxonomic revision of *Chironius bicarinatus* (Wied, 1820) (Serpentes: Colubridae), with description of a new species. *Herpetological Monographs* 34: 98–115.
- Fernandes DS, Hamdan B. 2014. A new species of *Chironius* Fitzinger, 1826 from the state of Bahia, northeastern Brazil (Serpentes: Colubridae). *Zootaxa* 3881: 563–575.
- Gonzales L, Reichle S, Entiauspe-Neto OM. 2020. A new species of *Oxyrhopus* Wagler, 1830 (Serpentes: Dipsadidae) from the Bolivian Andes. *Anais da Academia Brasileira de Ciências* 92: e20191428.
- Hamdan B, Fernandes DS. 2015. Taxonomic revision of *Chironius flavolineatus* (Jan, 1863) with description of a new species (Serpentes: Colubridae). *Zootaxa* 4012: 97–119.
- Hamdan B, Pereira AG, Loss-Oliveira L, Rödder D, Schrago CG. 2017. Evolutionary analysis of *Chironi-*

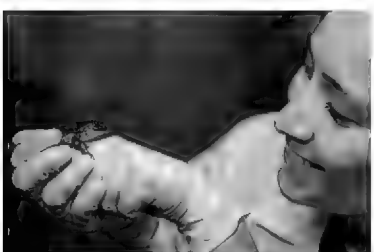
- us snakes unveils cryptic diversity and provides clues to diversification in the Neotropics. *Molecular Phylogenetics and Evolution* 116: 108–119.
- Herndon G, Dowling HG, Savage JM. 1960. A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. *Zoologica: Scientific Contributions of the New York Zoological Society* 45: 17–28.
- Hollis JL. 2006. Phylogenetics of the genus *Chironius* Fitzinger, 1826 (Serpentes, Colubridae) based on morphology. *Herpetologica* 62: 435–453.
- Ibisch PL, Mérida G. (Editors). 2003. *Biodiversidad: La Riqueza de Bolivia. Estado de Conocimiento y Conservación*. Ministerio de Desarrollo Sostenible, Editorial FAN, Santa Cruz de la Sierra, Bolivia. 638 p.
- Klaczko J, Montingelli GG, Zaher H. 2014. A combined morphological and molecular phylogeny of the genus *Chironius* Fitzinger, 1826 (Serpentes: Colubridae). *Zoological Journal of the Linnean Society* 171: 656–667.
- Kok PJR. 2010. A new species of *Chironius* Fitzinger, 1826 (Squamata: Colubridae) from the Pantepui region, northeastern South America. *Zootaxa* 2611: 31–44.
- Miller M, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA*. IEEE, Piscataway, New Jersey, USA.
- Navarro G, Maldonado M. 2004. *Geografía Ecológica de Bolivia*. Centro de Ecología Simón I. Patiño, Departamento de Difusión, Cochabamba, Bolivia. 122 p.
- Pesantes OS. 1994. A method for preparing the hemipenis of preserved snakes. *Journal of Herpetology* 28: 93–95.
- Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.
- Sudré V, Andrade-Junior A, Folly M, Azevedo JAR, Ávila RW, Curcio FF, Nunes PMS, Passos P. 2024. Revision of the *Chironius bicarinatus* complex (Serpentes: Colubridae): redefined species boundaries and description of a new species. *Vertebrate Zoology* 74: 85–120.
- Torres-Carvajal O, Koch C, Valencia JH, Venegas PJ, Echevarría LY. 2019a. Morphology and distribution of the South American snake *Chironius leucometapus* (Serpentes: Colubridae). *Phyllomedusa* 18: 241–254.
- Torres-Carvajal O, Echevarría LY, Lobos SE, Venegas PJ, Kok PJR. 2019b. Phylogeny, diversity, and biogeography of Neotropical Sipo Snakes (Serpentes: Colubrinae: *Chironius*). *Molecular Phylogenetics and Evolution* 130: 315–329.
- Vásquez R, Ibisch PL, Gerkmann B. 2003. Diversity of Bolivian Orchidaceae – a challenge for taxonomic, floristic, and conservation research. *Organisms, Diversity & Evolution* 3: 93–102.
- Wallach V, Williams KL, Boundy J. 2014. *Snakes of the World: a Catalogue of Living and Extinct Species*. CRC Press, Boca Raton, Florida, USA. 1,237 p.



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Teresa Camacho-Badani is a biologist born in Cochabamba, Bolivia, and has a M.Sc. in Conservation Biology. Her research is focused on the conservation and systematics of amphibians with a special interest in cloud forest and high Andean ecosystems. Currently she is working on the molecular systematics and monitoring of threatened amphibian and reptile species in Bolivia.

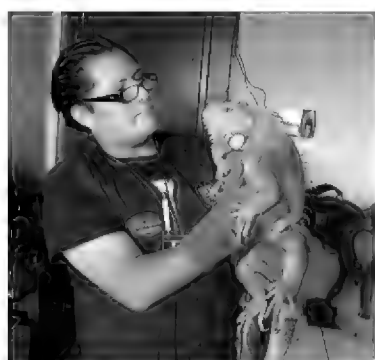
A New Species of Sipo Snake, *Chironius*



Rodrigo Aguayo is a Bolivian herpetologist and Research Associate at the Center for Biodiversity and Genetics (CBG) of the Universidad Mayor de San Simón in Bolivia. He works on the biodiversity, systematics, and conservation of amphibians and reptiles of Bolivia, mainly in the Andes and Amazonia. He is an author or co-author of more than 50 scientific articles in journals, notes, books, and book chapters. He has described 12 species new to science from Bolivia and Peru. A founder and board member of the Bolivian Association of Herpetology (ABH) and member of the Amphibian Specialist Group (ASG) of the IUCN Species Survival Commission, he is currently working as a consultant in environmental studies.



Rene Carpio-Real is a Biologist who has been working with amphibians and reptiles for several years. He has gained experience working in research and conservation, and has been monitoring different species of threatened amphibians and reptiles in the cloud forests (Yungas) of Bolivia. He is a member of the Bolivian Amphibian Initiative (BAI) and associate researcher at the Center for Biodiversity and Genetics (CBG) of the Universidad Mayor de San Simón.



Edson Pérez is a Biologist and Wildlife Veterinarian. His work focuses on wildlife conservation, and he is responsible for controlling wildlife trafficking in the municipality and the Government of Cochabamba, Bolivia. As a wildlife veterinarian, he carries out sanitary control of amphibians and reptiles as a specialist in the area of herpetology.



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Omar Torres-Carvajal graduated in Biological Sciences from Pontificia Universidad Católica del Ecuador (PUCE) in 1998, and in 2001 received a Master's degree in Ecology and Evolutionary Biology from the University of Kansas under the supervision of Dr. Linda Trueb. In 2005, Omar received a Ph.D. degree from the same institution with a thesis entitled "Phylogenetic systematics of South American lizards of the genus *Stenocercus* (Squamata: Iguania)." Between 2006 and 2008 Omar was a Postdoctoral Fellow at the Smithsonian Institution, National Museum of Natural History, Washington, DC, USA, working under the supervision of Dr. Kevin de Queiroz. Omar is currently Curator of Reptiles at the Zoology Museum (QCAZ) of PUCE and a Full Professor in the Department of Biology, PUCE. He is also a member of the Ecuadorian Academy of Sciences. Omar has published over 100 peer-reviewed scientific papers on the taxonomy, systematics, and biogeography of South American squamates, including Galápagos lizards. He has collaborated in the description of over 60 species of South American reptiles. Omar is mainly interested in the theory and practice of phylogenetic systematics and biogeography, particularly as they relate to the evolutionary biology of lizards and snakes.

Appendix 1: Additional material examined. These additional specimens are deposited at Museo de Historia Natural Alcide d'Orbigny (MHNC-R), Swedish Museum of Natural History [Naturhistoriska Riksmuseet] (NRM), Centro de Investigación de Recursos Acuáticos (CIRA), and Estación Biológica de Doñana (EBD).

Chironius fuscus: Asia (error): NRM 34 (holotype; digital photographs). **Bolivia:** *Beni*: Yacuma, CIRA 257; *La Paz*: Abel Iturralde, CIRA 463; CIRA 465; *Pando*: Abuná, CIRA 814 (digital photographs); *Cochabamba*: Chapare, EBD 23615H. Venezuela: Amazonas, EBD 20665H.

Chironius monticola: **Bolivia:** *Cochabamba*: Chapare, EBD 30128H; unknown locality, MHNC-R 3181.

Chironius flavolineatus: **Bolivia:** Ichilo, MHNC-R 384.

Chironius laurenti: **Bolivia:** *Beni*: Ballivian, Río Negro, MHNC-R 339.

Chironius leucometapus: **Ecuador:** *Morona Santiago*: Concesión Minera Kinross-Aurelian, DHMECN 10207; Taisha, Mutintsa, FHGO 1147, 3570; *Napo*: Cotundo, 12 km NW Reserva Ecológica Antisana, QCAZ 10977; *Pastaza*: Ingaru community, Reserva Privada Ankaku, QCAZ 9955; Bosque Protector Pablo López de Oglan Alto, DHMECN 3096; *Sucumbios*: San Pablo de Kantesiya, DHMECN 0087. NO FURTHER LOCALITY DATA: Piso tropical oriental, EPN 9552; Oriente, EPN13223. **Peru:** *Huánuco*: Cruz Blanca, Parque Nacional Tingo Maria, CORBIDI 15562; Pampamarca-Embalse, CORBIDI 16478; Rupa rupa, CORBIDI 16386; Sharco, CORBIDI 19374; *Pasco*: Puesto de Control Huampal, CORBIDI 7260; *San Martín*: Paitoja, CORBIDI1276; El Dorado, near Río Blanco, CORBIDI 01256; La Cueva, CORBIDI 0620.



Reproductive ecology, free-swimming tadpoles, and natural history of the Santa Marta Rocket Frog “*Colostethus*” *ruthveni* Kaplan, 1997 (Anura: Dendrobatidae), with a review of the distribution of larval and reproductive characters in Dendrobatoidea

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Abstract.—This study focuses on various biological aspect of the Santa Marta Rocket Frog, “*Colostethus*” *ruthveni*, from the Sierra Nevada de Santa Marta, in the Colombian Caribbean, and provides quantitative data on relative abundance and habitat use, and a comprehensive description of the free-swimming tadpoles and reproductive strategies. Additionally, the distribution of larval and reproductive characters across Dendrobatoidea was examined aiming to enhance the diagnostic criteria for the “*C.*” *ruthveni* group. A total of 853 specimens of “*C.*” *ruthveni* were recorded in six localities, indicating a relatively high abundance at each site. Leaf-litter and rocks were the most predominant substrates, although their proportions of use varied across the sites. The external morphology of free-swimming tadpoles of “*C.*” *ruthveni* resembles the former members of the *Colostethus sensu lato*. Courtship is a complex interaction of acoustic, visual, and tactile displays, which leads to cephalic amplexus. “*Colostethus*” *ruthveni* showed wide variation in oviposition sites, including both natural and artificial substrates at ground level, as well as creeping vegetation above the ground or water. This variability suggests the presence of multiple reproductive modes, one of them previously unknown for anurans. Several larval and reproductive characters of “*C.*” *ruthveni* consist of a compendium of ancestral states. However, some characters are of special interest because they could be considered reversions to ancestral states or apomorphies, which could constitute synapomorphies of the “*C.*” *ruthveni* group if they can be demonstrated to occur in the other undescribed members of the complex. Moreover, this study highlighted a distinctive trait of “*C.*” *ruthveni* as the only Dendrobatinae with tadpoles exhibiting a considerably narrow A-2 gap. The morphological and natural history information in this study provides insights into the evolutionary processes of poison frogs and basic information necessary for the management of this potentially endangered species.

Keywords. Character mapping, courtship, larval morphology, microhabitat uses, relative abundance, reproductive mode

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Resumen.—Investigamos varios aspectos de la biología de la rana cohete de Santa Marta “*Colostethus*” *ruthveni* de la Sierra Nevada de Santa Marta, Caribe colombiano. Se cuantificó la abundancia relativa y el uso del hábitat. Igualmente, se describieron los renacuajos de natación libre y las estrategias reproductivas. Además, se examinó la distribución de caracteres larvales y reproductivos a través de Dendrobatoidea como una aproximación a su evolución y para identificar si estos pueden complementar el diagnóstico del grupo “*C.*” *ruthveni*. Registramos 853 especímenes de “*C.*” *ruthveni* en seis localidades, lo que resultó en una abundancia

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relativa bastante alta en cada sitio. La hojarasca y las rocas fueron los sustratos más utilizados, aunque su proporción de uso varió según los lugares. La morfología externa de los renacuajos de “*C.*” *ruthveni* que nadan libremente se asemeja a la de los antiguos miembros de *Colostethus sensu lato*. El cortejo es una compleja interacción de exhibiciones acústicas, visuales y táctiles, que conducen al amplexo cefálico. “*Colostethus*” *ruthveni* mostró una amplia variación en los lugares de ovoposición, incluidos sustratos naturales y artificiales a nivel del suelo, así como vegetación rastrera sobre el suelo o el agua. Por lo tanto, consideramos que la especie exhibe múltiples modos reproductivos, uno de ellos desconocido hasta ahora para los anuros. Varios caracteres larvarios y reproductivos de “*C.*” *ruthveni* consisten en un compendio de estados ancestrales. Sin embargo, algunos caracteres son de especial interés porque podrían considerarse reversiones a estados ancestrales o apomorfías, que podrían constituir sinapomorfías del grupo “*C.*” *ruthveni* si se demuestra que ocurren en los otros miembros no descritos del complejo. Además, evidenciamos que los “*C.*” *ruthveni* es el único Dendrobatinae cuyos renacuajos poseen un espacio A-2 considerablemente estrecho. Toda esta información sobre morfología e historia natural permite comprender mejor los procesos evolutivos de las ranas venenosas y proporciona información básica necesaria para la gestión de esta especie potencialmente amenazada.

Palabras Claves. Abundancia relativa, cortejo, mapeo de caracteres, modo reproductivo, morfología larval

Introduction

Poison frogs of the superfamily Dendrobatoidea are one of the most representative groups of Neotropical amphibian fauna. These frogs have a series of highly diverse and particular characteristics including their color patterns, the presence or absence of toxicity, elaborate displays of visual and acoustic signals, and reproductive modes (Bourne et al. 2001; Caldwell and Lima 2003; Hill et al. 2011; Juncá et al. 1994; Lima and Keller 2003; Summers and Tumulty 2014; Wells 1980a,b,c; Weygoldt 1987). Within this family is the non-monophyletic genus *Colostethus sensu lato*, and while multiple efforts have been made to order it in recent decades (Grant 1998; Grant et al. 2006, 2017; Marin et al. 2018; Santos et al. 2009), this genus remains polyphyletic (Grant et al. 2017; Portik et al. 2023). The “*Colostethus*” *ruthveni* group *sensu* Grant et al. (2017) is more closely related to the aposematic dendrobatids (subfamily Dendrobatinae) than to *Colostethus sensu stricto* (ss; subfamily Colostethinae). Therefore, the “*C.*” *ruthveni* group constitutes an undescribed genus endemic to the Sierra Nevada de Santa Marta (SNSM), an isolated massif of the northeastern Caribbean coast of Colombia (Grant et al. 2017). Currently, the “*C.*” *ruthveni* group comprises the nominal species (“*Colostethus*” *ruthveni* Kaplan 1997 ss) and at least two undescribed species (Grant et al. 2017; Jiménez-Bolaño et al. 2019). “*Colostethus*” *ruthveni* ss was defined by Jiménez-Bolaño et al. (2019) as a population of the complex distributed in the northwestern sector of the SNSM, based on the bioacoustic variation, external morphology, and geographic location of the type locality.

In the last decade, our knowledge of the biology of the “*C.*” *ruthveni* group has increased considerably, including data on its distribution, feeding ecology, phylogenetic relationships, and acoustic repertoire (Blanco-Torres et al. 2014; González-Maya et al. 2011; Granda-Rodríguez et al. 2014; Grant et al. 2017; Jiménez-Bolaño et al. 2019; Meza-Joya et al. 2019).

However, multiple topics related to its reproductive mode are still unknown. Understanding the reproductive modes is of great interest, because natural and sexual selection favor reproductive mechanisms that maximize the probability of successful mating and the survival of offspring, thereby directly affecting fitness and biological performance in response to different selective pressures (Carvajal-Castro et al. 2020). In this sense, the reproductive mode can be defined as a combination of ecological, physiological, developmental, and behavioral traits such as the oviposition site, ovule morphology, clutch size, and the presence or absence of different types of parental care (Duellman and Trueb 1994). In the Dendrobatoidea, the study of the reproductive modes is particularly interesting due to their great diversity (Carvajal-Castro et al. 2020; Vitt and Caldwell 2014). However, the only information available on this aspect for the group “*C.*” *ruthveni* was published more than a century ago by Ruthven and Gaige (1915), who briefly described the reproductive habitat and microhabitat, and the male transport of larvae.

Tadpoles of “*C.*” *ruthveni* present the typical morphology of *Colostethus sensu lato* larvae. The first tadpole description was based on back-riding larvae removed from the male dorsum and free-swimming specimens (Ruthven and Gaige 1915). However, this description is too limited by current standards and describes the free-swimming and back-riding tadpoles together. This approach is not recommended, as it makes comparisons difficult due to the ontogenetic variations between the different larval stages (Anganoy-Criollo 2013). More recent research has been limited to describing characters, with the aim of inferring phylogenetic relationships based on larval morphology and the evolution of larval characters (Anganoy-Criollo and Cepeda-Quilindo 2017; Sánchez 2013). Such studies revealed that “*C.*” *ruthveni* apparently possesses multiple ancestral character states (e.g., the presence of a notch in the upper jaw sheath, the long gut sinistrally coiled, and the inner margin of the nasal rim projected), especially

considering the phylogenetic relationships of the group *sensu* Grant et al. (2017). Given our limited knowledge of the external morphology of free-swimming tadpoles of “*C.*” *ruthveni* and the potential phylogenetic implications obtained from this line of evidence, a redescription of the tadpoles of the species following the standards of current descriptions is necessary.

This species is currently categorized by the IUCN as Near Threatened (NT- B1a; IUCN SSC Amphibian Specialist Group 2018) due to habitat loss because of agricultural activities (including pollution) and livestock, particularly in the southeastern sector of the SNSM (Granda-Rodríguez et al. 2020). Despite its relatively small range (EOO < 11,000 km²), the populations appear to be stable and somewhat tolerant to disturbance. Although the IUCN categorization states that populations are stable and abundant, that statement is based on anecdotal observations that do not account for sampling effort. Consequently, acquiring information on the basic ecological dimensions of “*C.*” *ruthveni*, its relative abundance, and habitat use will provide more appropriate tools to identify priority areas for the management and conservation of this species. Thus, according to the multiple knowledge gaps that exist on the biology of the “*C.*” *ruthveni* group, this study was developed with the following objectives: (1) quantifying the relative abundance and use of microhabitats; (2) redescriving the external morphology of free-swimming tadpoles; (3) documenting some aspects of the reproductive mode, such as amplexus and laying site, in a population of *Colostethus ruthveni* ss; and (4) exploring from a phylogenetic perspective the distribution of external larval and reproductive characters and how these can potentially facilitate the diagnosis of the group “*C.*”

ruthveni based on the most recent available topology of Dendrobatoidea.

Materials and Methods

Study Area

This study was carried out at 10 sites distributed in four pericontinental basins in the distribution area of “*C.*” *ruthveni* ss, the northwestern sector of the Sierra Nevada de Santa Marta, district of Santa Marta, department of Magdalena, Colombian Caribbean (Fig. 1, Table 1). The sites occupy the altitudinal strip from the foothills to the low mountains, at altitudes between 400 and 1,521 m, which cover almost the entire altitudinal distribution known for “*C.*” *ruthveni* (Granda-Rodríguez et al. 2014; Jiménez-Bolaño et al. 2019). In its lower sector, this altitudinal strip includes the tropical dry biomes that succeed one another with the sub-Andean Forest biome at approximately 700 m (Fundación Pro-Sierra Nevada 1998), except for the Los Rodríguez stream basin, where the limit could be at around 550 m asl. Its presence at the lower altitude is due to the fog, a product of the condensation due to the humid coastal winds (Fundación Pro-Sierra Nevada 1998). The area’s water regimes vary between unimodal and bimodal, with one or two annual rainfall peaks. Both climatic modes converge from the driest period, extending from December to March, and the rainiest period, extending from August to November. There may or may not be a drop in rainfall in July. Mountain lotic ecosystems show rapid flow due to the steep slope of the mountain, and have abundant boulders and sandy or pebble-covered bottoms (Manjarrés-García and Manjarrés-Pinzón 2004).

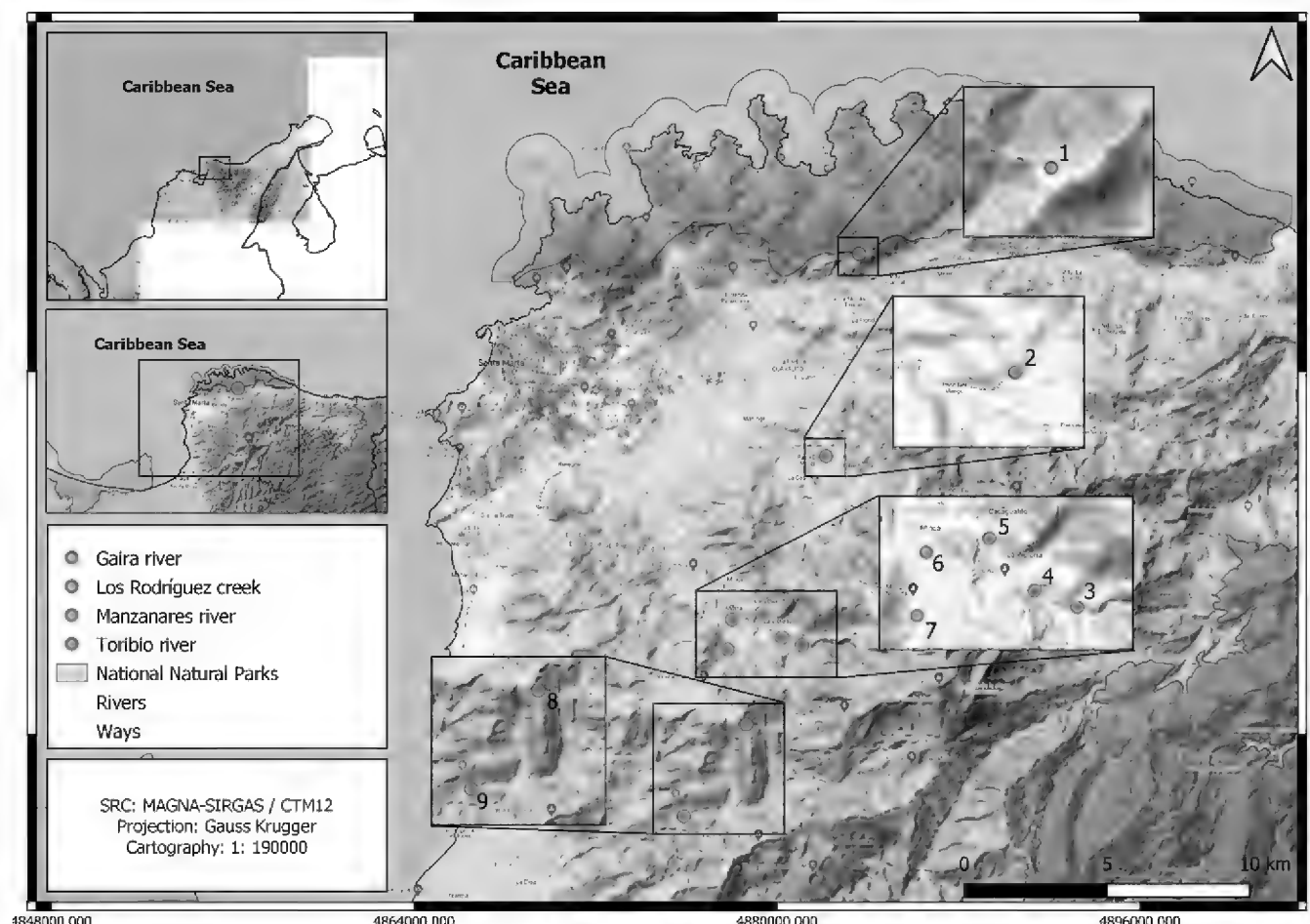


Fig. 1. Localization of the study area. 1. Las Tinajas, 2. Paso El Mango, 3. Sierra Minca, 4. La Victoria, 5. Pozo Azul, 6. Arimaca, 7. Marinca, 8. Bella Vista, 9. Central Córdoba. Data of reproductive biology were from 2, 4, 5–9. Data of relative abundance and microhabitat were from 1–4. Tadpole batch CBUMAG:ANF:00662 from 4. Developed by Juan David Jiménez-Bolaño.

Relative Abundance and Microhabitat Use

To estimate the relative abundance, 21 sampling events were carried out at six locations during the rainy and dry seasons between 2005 and 2021 (Tables 1 and 2). Two observers searched randomly, carefully inspecting the

microhabitats to find specimens of “*C.*” *ruthveni* using a visual encounter survey method (Crump and Scott 1994). The sampling effort varied between 2–6 hours (h) per event, for a total of 105 h (Table 1). Based on the total counts of individuals, a relative abundance index (RAI) was estimated, defined as the number of individuals (n)

Table 1. List of localities where “*Colostethus*” *ruthveni sensu stricto* was recorded, with date of observation, sampling effort, total count per sampling event, and relative abundance (n/h/obs) per sampling event.

Basin and site	Sampling date	Sampling effort (h × obs)	n	RAI (n/h/obs)
Gaira river				
La Victoria	7/11/2005	5 × 2	37	3.7
	12/11/2005	5 × 2	43	4.3
	21/03/2010	5 × 2	17	1.7
	22/03/2010	5 × 2	20	2.0
	06/06/2010	5 × 2	30	3.0
	07/06/2010	5 × 2	38	3.8
	06/07/2011	5 × 2	24	2.4
Sierra Minca	20/10/2020	5 × 2	35	3.5
	21/10/2020	5 × 2	44	4.4
	22/10/2020	5 × 2	46	4.6
	24/01/2021	4 × 2	80	10.0
Manzanares river				
Paso el Mango	15/04/2011	2 × 2	10	2.5
Los Rodríguez Creek				
Las Tinajas	10/02/2006	6 × 2	42	3.5
	20/02/2007	6 × 2	55	4.6
	20/02/2010	6 × 2	68	5.7
	06/07/2011	6 × 2	28	2.3
	18/08/2014	2 × 2	20	5.0
	17/01/2021	4 × 2	45	5.6
Toribio river				
Bella Vista	5/10/2014	2 × 2	11	2.8
Central Córdoba	26/11/2020	5 × 2	63	6.3
	27/11/2020	5 × 2	35	3.5
	28/11/2020	5 × 2	62	6.2

Table 2. Microhabitat use of “*Colostethus*” *ruthveni sensu stricto* in four localities of the northwestern sector of the Sierra Nevada de Santa Marta. χ^2 = Chi square test, df = degrees of freedom.

Site	Bare floor	Boulders	Decayed logs	Leaf-litter	Leaves	Total	χ^2
Las Tinajas	14	52	4	11	4	11	96
La Victoria	3	9	4	13	3	5	37
Sierra Minca	2	7	2	22	2	15	50
Central Córdoba	1	9	5	11	1	3	30
Total	20	77	15	57	10	34	213

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*

Table 3. Summary of morphometric characters of free-swimming tadpoles of “*Colostethus*” *ruthveni sensu stricto* through the Gosner (1960) stages of s25 through s38. Measurements are expressed in mm, with range (average ± standard deviation). Abbreviations: total length (TL), body length (BL), tail length (TAL), body height (BH), maximum tail height (MTH), tail muscle height (TMH), nostril-snout distance (NSD), nostril-eye distance (NED), nostril length (NL), eye diameter (ED), spiracle height (SH), spiracle length (SL), spiracle-snout distance (SSD), eye-snout distance (ESD), body width (BW), internarial distance (IND), interorbital distance (IOD), tail muscle width (TMW), oral disc width (ODW), anterior lip gap width (AL-gap), width of medial gap in second anterior tooth row (A-2 gap), upper jaw sheath width (UJS-W), lower jaw sheath width (LJS-W), papillae in the anterior per mm (P-AL/mm), and papillae in the posterior lip per mm (P-PL/mm).

Character	s25 (n=15)	s26 (n=13)	s27 (n=8)	s28 (n=5)	s29 (n=2)	s30–s31 (n=2)	s33 (n=1)	s34 (n=3)	s35 (n=3)	s36 (n=2)	s37 (n=1)	s38 (n=2)
TL	12.9–17.6 (15.6±1.5)	16.7–23.3 (18.8±1.8)	23.8–27.7 (25.8±1.6)	27.2–29.2 (28.1±0.1)	28.6–29.6 (29.1±0.7)	28.5–29.4 (29.0±0.6)	28.5	29.6–30.5 (30.2±0.5)	29.3–31.5 (30.5±1.1)	31.8–32.3 (32.0±0.3)	30.6	32.5–33.8 (33.1±0.9)
BL	5.0–6.7 (6.0±0.6)	6.5–8.4 (7.1±0.7)	9.0–10.2 (9.6±0.5)	10.0–10.9 (10.3±0.4)	10.3–10.6 (10.4±0.2)	10.2–10.3 (10.3±0.1)	10.4	10.6–11.0 (10.8±0.2)	10.0–11.1 (10.7±0.6)	10.7–11.1 (10.9±0.3)	10.7	11.1–11.6 (11.3±0.3)
TAL	7.9–10.9 (9.7±0.9)	10.1–14.9 (11.6±1.7)	14.8–17.5 (16.3±1.2)	17.1–18.7 (17.8±0.7)	18.0–19.3 (18.6±0.9)	18.3–19.1 (18.7±0.6)	18.1	19.0–19.5 (19.3±0.3)	19.3–20.4 (19.8±0.5)	21.1–21.2 (21.1±0.1)	19.9	21.4–22.2 (21.8±0.6)
BH	2.8–3.7 (3.3±0.3)	3.6–4.6 (4.0±0.4)	4.2–5.3 (4.9±0.4)	4.9–5.9 (5.3±0.4)	5.2–5.4 (5.3±0.1)	3.3–5.2 (4.3±1.3)	5.3	5.4–5.7 (5.6±0.2)	5.4–5.5 (5.5±0.1)	5.5–5.6 (5.5±0.1)	5.4	5.2–5.5 (5.3±0.2)
MTH	2.4–3.2 (2.8±0.2)	3.0–4.1 (3.4±0.3)	4.0–4.6 (4.4±0.2)	4.5–4.9 (4.7±0.1)	4.6–4.7 (4.6±0.1)	4.3–4.5 (4.4±0.1)	4.9	4.6–5.1 (4.9±0.2)	4.5–5.0 (4.7±0.3)	4.9–5.1 (5.0±0.1)	4.7	4.9–5.0 (4.9±0.1)
TMH	1.3–1.9 (1.7±0.2)	1.8–2.4 (2.0±0.9)	2.8–4.5 (3.7±0.7)	3.1–3.6 (3.3±0.2)	3.3–3.5 (3.4±0.1)	3.0–3.3 (3.2±0.2)	3.4	3.2–3.7 (3.5±0.3)	3.3–3.6 (3.5±0.1)	3.3–3.5 (3.4±0.1)	3.2	3.0–3.2 (3.1±0.1)
NSD	0.5–0.7 (0.6±0.1)	0.6–0.9 (0.7±0.1)	0.9–1.2 (1.0±0.1)	1.1–1.3 (1.2±0.1)	1.0–1.1 (1.0±0.1)	1.0–1.1 (1.1±0.1)	1.1	0.9–1.1 (1.0±0.1)	1.0–1.2 (1.1±0.1)	0.9–1.0 (0.9±0.1)	0.9	0.9–1.1 (1.0±0.1)
NED	0.4–0.7 (0.6±0.1)	0.6–0.9 (0.7±0.1)	0.9–1.0 (1.0±0.05)	0.9–1.0 (1.0±0.05)	1.0–1.0 (1.0±0.0)	0.9–1.0 (1.0±0.1)	1.0	0.9–1.0 (1.0±0.1)	0.8–1.0 (0.9±0.1)	0.9–0.9 (0.9±0.0)	0.8	1.0–1.0 (1.0±0.0)
NL	0.1–0.2 (0.1±0.05)	0.2–0.3 (0.2±0.03)	0.2–0.4 (0.3±0.05)	0.3–0.4 (0.3±0.05)	0.3–0.3 (0.3±0.0)	0.3–0.4 (0.4±0.1)	0.2	0.4–0.5 (0.4±0.1)	0.3–0.4 (0.4±0.1)	0.4–0.4 (0.4±0.0)	0.3	0.3–0.4 (0.3±0.1)
ED	12.9–17.6 (15.6±1.5)	0.6–0.9 (0.8±0.1)	0.8–1.1 (0.9±0.1)	1.0–1.1 (1.0±0.05)	1.1–1.2 (1.1±0.1)	1.1–1.1 (1.1±0.0)	1.2	1.2–1.3 (1.3±0.1)	1.3–1.5 (1.4±0.1)	1.3–1.3 (1.3±0.0)	1.4	1.4–1.5 (1.4±0.1)
SH	0.4–0.7 (0.5±0.09)	0.6–0.8 (0.6±0.1)	0.6–1.2 (0.9±0.2)	0.9–1.2 (1.0±0.1)	1.3–1.4 (1.3±0.1)	1.0–1.3 (1.2±0.2)	1.3	1.0–1.3 (1.2±0.1)	1.0–1.3 (1.1±0.2)	1.2–1.3 (1.2±0.1)	1	0.9–1.1 (1.0±0.1)
SL	0.8–1.3 (1.1±0.1)	1.1–1.6 (1.3±0.1)	1.4–2.7 (2.0±0.4)	1.6–2.5 (2.1±0.3)	2.3–2.7 (2.5±0.3)	1.5–2.7 (2.1±0.8)	2.7	2.1–2.5 (2.3±0.2)	2.1–2.4 (2.3±0.1)	2.2–2.3 (2.2±0.1)	1.9	2.0–2.4 (2.2±0.2)
SSD	3.2–4.3 (3.9±0.4)	4.2–5.1 (4.5±0.3)	5.7–6.6 (6.1±0.3)	6.5–6.8 (6.6±0.1)	6.4–6.5 (6.4±0.1)	6.4–6.4 (6.4±0.0)	6.8	6.6–6.7 (6.6±0.1)	6.6–7.0 (6.8±0.2)	6.6–6.8 (6.7±0.1)	6.7	6.5–7.1 (6.8±0.4)
ESD	1.2–1.6 (1.4±0.2)	1.5–1.9 (1.7±0.1)	2.2–2.5 (2.3±0.1)	2.5–2.6 (2.5±0.05)	2.2–2.5 (2.3±0.2)	2.3–2.3 (2.3±0.0)	2.2	2.2–2.3 (2.2±0.1)	2.1–2.6 (2.3±0.3)	2.1–2.2 (2.1±0.1)	2	2.3–2.5 (2.4±0.1)
BW	3.8–5.6 (4.6±0.6)	4.6–6.4 (5.4±0.5)	5.6–6.4 (6.0±0.3)	6.0–6.5 (6.4±0.2)	6.3–6.5 (6.4±0.1)	6.0–6.4 (6.2±0.3)	6.5	6.8–7.1 (6.9±0.1)	6.4–6.9 (6.7±0.2)	6.9–6.9 (6.9±0.0)	6.8	6.6–6.8 (6.7±0.1)
IND	1.1–1.6 (1.4±0.1)	1.3–1.8 (1.6±0.1)	1.8–2.0 (1.9±0.05)	1.9–2.0 (1.9±0.05)	1.9–2.1 (2.0±0.1)	1.9–2.0 (2.0±0.1)	2.1	2.0–2.1 (2.0±0.1)	2.0–2.1 (2.1±0.03)	2.0–2.1 (2.0±0.1)	2.1	2.1–2.1 (2.1±0.0)
IOD	1.6–2.2 (1.9±0.2)	2.0–2.5 (2.2±0.1)	2.5–2.8 (2.7±0.1)	2.8–2.9 (2.8±0.04)	2.9–2.9 (2.9±0.0)	2.9–2.9 (2.9±0.0)	3.1	3.2–3.3 (3.3±0.1)	3.1–3.3 (3.2±0.1)	3.3–3.3 (3.3±0.0)	3.4	3.5–3.5 (3.5±0.0)
TMW	1.3–2.1 (1.7±0.2)	1.7–2.7 (2.1±0.3)	2.2–3.2 (2.8±0.3)	2.8–3.3 (3.1±0.1)	3.0–3.2 (3.1±0.1)	3.0–3.1 (3.1±0.1)	3.1	3.2–3.5 (3.4±0.1)	3.4–4.0 (3.6±0.4)	3.6–3.7 (3.6±0.1)	3.4	3.5–3.6 (3.0±0.1)
ODW	1.7–2.9 (2.2±0.4)	2.1–3.0 (2.5±0.2)	2.7–3.0 (2.9±0.1)	2.9–3.4 (3.1±0.2)	3.0–3.8 (3.4±0.6)	2.7–2.9 (2.8±0.1)	3.2	3.2–3.5 (3.3±0.2)	2.9–3.1 (3.0±0.1)	3.1–3.2 (3.1±0.1)	3.3	3.0–3.3 (3.1±0.2)
AL gap	0.9–1.4 (1.1±0.6)	1.1–1.6 (1.3±0.1)	1.4–1.7 (1.5±0.1)	1.3–1.7 (1.5±0.1)	1.5–1.6 (1.5±0.1)	1.4–1.8 (1.6±0.3)	1.7	1.5–1.8 (1.7±0.1)	1.6–2.1 (1.8±0.3)	1.4–1.7 (1.5±0.2)	1.8	1.6–1.7 (1.6±0.1)
UJS-W	0.6–1.0 (0.9±0.1)	0.8–1.2 (1.0±0.1)	1.2–1.5 (1.3±0.1)	1.3–1.5 (1.4±0.1)	1.3–1.4 (1.3±0.1)	1.3–1.4 (1.4±0.1)	1.4	1.3–1.5 (1.4±0.1)	1.4–1.6 (1.5±0.1)	1.3–1.5 (1.4±0.1)	1.5	1.3–1.4 (1.3±0.1)
LJS-W	0.4–0.7 (0.5±0.1)	0.4–0.7 (0.6±0.1)	0.8–1.0 (0.9±0.1)	0.9–1.1 (1.0±0.1)	0.9–1.0 (0.9±0.1)	0.8–0.8 (0.8±0.0)	1.0	0.9–1.1 (1.0±0.1)	1.1–1.2 (1.1±0.1)	1.0–1.1 (1.0±0.1)	1	1.0–1.0 (1.0±0.0)
P-AL/mm	11–17 (14.3±1.8)	15–16 (15.5±0.5)	15–18 (17.3±1.0)	18–19 (18.6±0.5)	14–15 (14.5±0.7)	14–20 (17.0±4.2)	17	19–19 (19.0±0.0)	16–17 (16.7±0.6)	16–20 (18.0±2.8)	23	15–18 (16.5±2.1)
P-PL/mm	12–21 (16.6±2.2)	14–18 (15.8±1.5)	13–16 (14.61±1.3)	13–20 (15.0±3.1)	12–13 (12.5±0.7)	11–14 (12.5±2.1)	20	15–16 (15.3±0.6)	13–18 (15.0±2.6)	14–17 (15.5±2.1)	21	13–15 (14.0±1.4)
A-2 gap	0.07–0.1 (0.1±0.02)	0.06–0.2 (0.1±0.04)	0.09–0.2 (0.1±0.05)	0.09–0.2 (1.0±0.05)	0.1–0.1 (0.1±0.0)	0.1–0.1 (0.1±0.0)	0.1	0.09–0.1 (0.1±0.01)	0.1–0.2 (0.2±0.1)	0.09–0.1 (0.1±0.01)	0.1	0.1–0.1 (0.1±0.0)

per capture effort (hours × observers) and denoted as n/h/obs (Lips 1999). The evaluation of microhabitat use followed Johnson (1980), and Krausman (1999), which identifies the specific components of habitat utilized and their respective proportions of use. The substrate

directly beneath each specimen was documented and the frequency of observations for each substrate type was calculated following Inger (1994) and Vargas-Salinas and Castro (1999). A Chi-square test of homogeneity (χ^2) was employed to assess potential variations in

substrate (microhabitat) usage. The null hypothesis states no significant differences among the observed categories. To ensure accuracy, the expected frequencies for each microhabitat category observed in the field were calculated, assuming equal probabilities of occupancy across microhabitats.

Free-swimming Tadpoles

This study followed the nomenclature of the Dendrobatoidea superfamily proposed by Grant et al. (2017), with the arrangements of Marin et al. (2018). The description of the tadpole was based on a group of 57 tadpoles deposited in the amphibian collection at the Centro de Colecciones Científicas de la Universidad del Magdalena (CBUMAG:ANF:00662), that had been collected by J. M. Renjifo at the La Victoria farm, Minca village, District of Santa Marta, department of Magdalena, Colombia (see Fig. 1). Additionally, some observations on the natural history were obtained in the localities of Las Tinajas, Bella Vista, Pozo Azul, and Arimaca. Tadpoles were determined to the species level as “*Colostethus*” *ruthveni sensu stricto* by the following three characteristics: (1) the presence of a golden band on the body of the tadpole described by Ruthven and Gaige (1915); (2) the geographic distribution of the complex proposed by Jiménez-Bolaño et al. (2019); and (3) the presence of three metamorphics in the group whose morphological characters correspond to those described by Kaplan (1997). A Leica M205A stereomicroscope with a Leica DFC450 camera was used for the examination and photography of the specimens. The morphometric characters (expressed in mm) were characterized with the Leica Application Suite software (Version 4.6.0), with a precision of ± 0.01 .

The tadpole description was based on the external morphology of three tadpoles at stage 35 of Gosner (1960), since this stage represents the midpoint of larval development (Suarez-Mayorga and Lynch 2001). The other stages were used to describe the ontogenetic variation of the morphological and morphometric characters. The terminology for morphometric characterization proposed by Altig and McDiarmid (1999) and Anganoy-Criollo (2013) was followed for evaluating the total length (TL), body length (BL), body width (BW), tail length (TAL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), internarial distance (IND), interorbital distance (IOD), oral disc width (ODW), anterior lip gap width (AL-gap), width of medial gap in second anterior tooth row (A-2 gap), nostril length (NL), nostril-snout distance (NSD), nostril-eye distance (NED), spiracle length (SL), spiracle height (SH), spiracle -snout distance (SSD), eye diameter (ED), eye-snout distance (ESD), upper jaw sheath width (UJS-W), and lower jaw sheath width (LJS-W). Additionally, the arrangement of the rows of papillae in the anterior (PAL) and posterior (PPL) lips were examined and described, following the

terminology of Sánchez (2010). The number of papillae on each lip was counted in the space of 1 mm (Anganoy-Criollo 2013). For metamorphic tadpoles, total length, tail length, and body width were measured.

Reproductive Ecology

To identify egg-laying sites, seven monthly fieldtrips lasting two days each were conducted in Las Tinajas from June to December 2015, as well as three additional fieldtrips during August 2015 (two days), December 2016 (one day), and January 2021 (one day) in Bella Vista, Arimaca, and Sierra Minca, respectively. At each site, two observers explored the watercourses by proceeding upstream and using the visual encounter survey method (Crump and Scott 1994). The searches lasted from 0600 to 1800 h for a total effort of 210 h \times 2 observers (168 h in Los Rodríguez ravine, 24 h in Bella Vista, 12 h in Marinca, and 6 h in Sierra Minca). At each egg laying site, the number of eggs, egg diameter, location, and distance from the water source were recorded. Information on courtship and type of amplexus was obtained through direct observations. Aspects of courtship such as color change, jumps, circle jumps, body elevation, and others, were described following the terminology of Hödl and Amézquita (2001). The reproductive mode was established from the proposal of Nunes-De-Almeida et al. (2021, see Supplemental Information S2), where 11 reproductive and larval characters were examined using a dichotomous key.

Distribution of Larval and Reproductive Characters

A matrix coding 35 phenotypic characters (larval and reproductive morphology) for each genus of the Dendrobatoidea superfamily (22 genera) was built. Thirty-two characters were extracted from Appendix 1 of Grant et al. (2017) (characters 94-126). The coding of these characters was extracted via an extensive literature review on the free-swimming tadpole morphology and reproductive strategies of Dendrobatoidea (Almendáriz et al. 2012; Anganoy-Criollo 2013; Anganoy-Criollo and Cepeda-Quilindo 2017; Barrio-Amorós et al. 2004; Breder 1946; Brown et al. 2008; Caldwell 2005; Caldwell and Myers 1990; Caldwell et al. 2002; Carvajal-Castro et al. 2020; Castillo-Trenn 2004; Dias et al. 2018a,b, 2021; Donnelly et al. 1990; Downie and Nokhbatolfoghahai 2014; Duarte-Marín et al. 2020; Duellman 2004; Edwards 1971, 1974; Fouquet et al. 2019; French et al. 2019; González-Maya et al. 2011; Grant 1998; Grant et al. 2006, 2007, 2017; Granda-Rodríguez et al. 2014; Granda-Rodríguez et al. 2018; Hill et al. 2011; Jiménez-Bolaño et al. 2019; Klein et al. 2020; Kok et al. 2006a,b, 2010, 2013; La Marca 1985, 1989, 1993, 1994; La Marca and Mijares-Urrutia 1988; Lehtinen and Halley 2008; Lescure 1976; Lima et al. 2014, 2015; Lüddecke 1999; Lynch 1982; Marin et al. 2018; Masche et al. 2010; Menin

et al. 2017; Mijares-Urrutia 1991; Mijares-Urrutia and La Marca 1997; Moraes et al. 2019; Myers and Donnelly 1997, 2001; Myers et al. 1984; Pezzuti et al. 2022; Páez-Vacas et al. 2010; Perez-Peña et al. 2010; Pisso-Florez et al. 2023; Poelman et al. 2010; Regnet et al. 2023; Rojas-Morales et al. 2021; Rodriguez and Myers 1993; Ruthven and Gaige 1915; Sánchez 2013; Savage 1968, 2002; Schulze et al. 2015; Schulte 1990; Serrano-Rojas et al. 2017; Silverstone 1975; Simões et al. 2010, 2013; Simões and Lima 2012; Summers and Tumulty 2014; Twomey and Brown 2008, 2009; van Wijngaarden and Bolaños 1992; Vitt and Caldwell 2014; von May et al. 2008; Wells 1980a,b; Weygoldt 1987).

One of the characters from Grant et al. (2006, 2017) was reinterpreted as follows:

119. Oviposition site: aquatic = 0; terrestrial: at ground level = 1; terrestrial: phytoteltama = 2; terrestrial: in leaves above ground or water level = 3.

This character will be considered as additive for describing the degree of association with the ground level or the distance from the water (Grant et al. 2006). State 1 includes all microhabitats at ground level such as leaf litter or bare soil, as well as on and under fallen objects such as rocks, logs, and even artificial objects. State 2 is restricted to cavities with liquid water in terrestrial plants. State 3 consists of species that lay their eggs on the upper surface or underside of the leaves, which had been considered as part of state 2 by Grant et al. (2006, 2017). Additionally, two new characters were declared, following the argument of Sereno (2007), that were enumerated following the consecutive numbering of the character list of Grant et al. (2017):

189. Second keratodon row of anterior lip, medial gap (A-2 gap): absent = 0; present = 1.

190. First keratodon row of posterior lip, medial gap (P-1 gap): absent = 0; present = 1.

Subsequently, these 35 characters were mapped by hand on the topology that summarizes the generic relationships of Dendrobatoidea proposed by Grant et al. (2017, modified from figure 47), with the goal of identifying how the larval morphological plans and the reproductive modes are distributed throughout this hypothesis of the evolutionary relationships of the Dendrobatoidea. In addition, we explored the intra- and intergeneric variation of a continuous character: the ratio of the width of the medial gap of the second row of keratodon teeth of the anterior lip by the width of the oral disc (A-2 gap/ODW %), since it has recently been proposed as potentially useful for diagnosing some taxa within Dendrobatoidea (Granda-Rodríguez et al. 2018).

Results

Relative Abundance and Microhabitat Use

A total of 853 individuals of “*C.*” *ruthveni* ss were observed in six study locations. The species was very abundant in all samplings and locations (Table 1), since the RAI in all cases reached values greater than 1, i.e., between 1.7–6.3 n/h/obs, with an exceptional observation of 10 n/h/obs. This means that the species maintained an approximate encounter rate of two to six individuals per hour throughout the entire time window studied. Although the largest numbers of individuals were detected in Las Tinajas (n = 258) and La Victoria (n = 209), the highest RAI values were obtained in Sierra Minca (10 n/h/obs) and Central Córdoba (6.2 n/h/obs), since less sampling effort was required to record considerable numbers of specimens at these sites. Microhabitat use data were obtained from 213 specimens. Six categories of substrate were used by the species: bare floor, boulders, decayed logs, leaf-litter, leaves, and stream, where the most heavily used substrates were boulders (77 individuals, 36.2%) and leaf-litter (57 individuals, 26.8%). Differential use of microhabitats was found at all sites, and the proportion of use of each microhabitat also varied between them (Table 2). In the locality of Las Tinajas, most individuals were found associated with boulders, demonstrating a statistically significant difference ($\chi^2 = 102.38$, $df = 5$, $p\text{-value} < 0.001$). In La Victoria, a greater use of leaf litter and boulders was found ($\chi^2 = 13.1$, $df = 5$, $p\text{-value} = 0.022$). In Sierra Minca, the highest usage occurred in leaf litter and individuals in streams ($\chi^2 = 42.4$, $df = 5$, $p\text{-value} < 0.001$). Finally, in Central Córdoba, a greater usage of leaf litter was found ($\chi^2 = 17.6$, $df = 5$, $p\text{-value} < 0.001$).

Redescription of Free-swimming Tadpoles

Table 3 summarizes the morphometric characters analyzed, with the individual data for each tadpole presented in Supplementary Material 1: Table S1. Stage 35 tadpoles have the following characteristics: body ovoid in dorsal view and compressed in lateral view (BW/BL = 60–64%, BH/BW = 78–86%, Fig. 2), reaching just over one-third of the total length (BL/TL = 34–36%); snout rounded in dorsal and lateral views; nostrils rounded in dorsal view, directed dorsolaterally, with gently projecting margins, equidistant between eyes and tip of snout, occupying almost one-third of eye diameter (NL/ED = 21–31%); internarial distance approximately two-thirds of the interorbital distance (IND/IOD = 62–67%); and the interorbital distance one-half the body width (IOD/BW = 47–48%). Eyes located dorsally and directed dorsolaterally, with a diameter less than half the interorbital distance (ED/IOD = 39%). Sinister spiracle, as long as high (SH/SL = 48–54%), visible in lateral, dorsal, and ventral views, directed

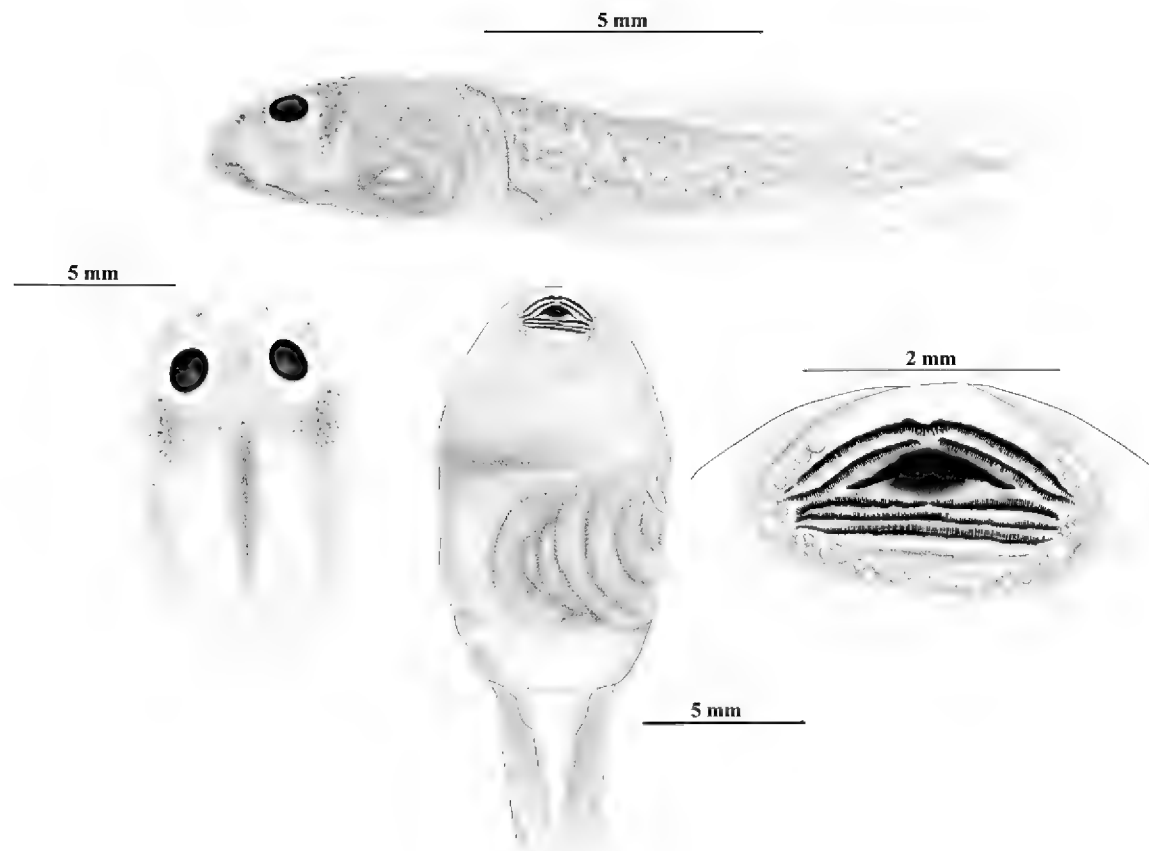


Fig. 2. Detail of larval morphology of a free-swimming tadpole of “*Colostethus ruthveni*” *sensu stricto* at stage 35 (*sensu* Gosner 1960) from the batch CBUMAG:ANF:00662. Drawings by Jhuliyana Lopez-Caro.

posteriodorsally, opening posterior to midpoint of body, exceeding half the body length ($SSD/TL = 61\text{--}66\%$).

Vent opening dexterous, tail musculature as long as the fin, fin tip rounded. The tail exceeds half the total length ($TAL/TL = 64\text{--}66\%$) and its maximum height is 15–16% of the total length, but 82–93% of the body height. Caudal myotomes visible, tail axis straight. Robust caudal musculature in the front part of the body, tapering gradually toward the end of the tail. The caudal musculature is half as wide and about one-third shorter than the body ($TMW/BW = 51\text{--}58\%$, $TMH/BH = 61\text{--}65\%$). The dorsal fin extends only as a slight ridge along the body at the body-tail junction and is narrower than the tail muscle. In the middle of the tail, the dorsal fin is slightly higher than the ventral fin. The ventral fin originates from the body and is narrower than the tail muscle. The point of origin of the hind legs is parallel to the cloacal tube.

The oral disc is emarginate, directed anteroventrally, surrounded by marginal papillae, (except in the middle of the anterior lip, $AL\text{-}gap/ODW = 53\text{--}68\%$) occupying just under half the width of the body ($ODW/BW = 43\text{--}48\%$). Papillae are abundant on both lips, where the right lateral margin of the anterior lip has 16–17 papillae/mm that are blunt and smaller than those of the posterior lip. On the posterior lip, the papillae in the central part are conical, elongated, and blunt (13–16 papillae/mm), while on the lateral margins they are conical and pointed. Dental formula 2(2)/3(1) with a very small A-2 gap ($A\text{-}2\text{ gap}/ODW = 3\text{--}7\%$). The first row of teeth of the posterior lip (P-1) is briefly interrupted (P-1 gap), while P-2 and P-3 are continuous. Rows P-1 and P-2 are of equal length, while P-3 is slightly shorter. The teeth in P-3 are slightly smaller and less keratinized than those in the other rows. The upper jaw sheath is half the width of the oral disc ($UJS/ODW = 48\text{--}52\%$) with thin, arch-shaped, medially

notched (W-shaped) lateral processes. On the other hand, the lower jaw sheath is narrower ($LJS/ODW = 37\text{--}39\%$), thinner, and broadly V-shaped; both with small, blunt striae.

The general morphology of the free-swimming tadpoles did not present abrupt changes between the different stages analyzed (Fig. 3a), although slight variations were observed in some characters of the configuration of the oral disc. In stages 25–38, only one row of papillae was observed on the anterior lip and the number of these varied in a range of 11–23 papillae/mm. On the posterior lip, a simple row, a biseriata row, and two rows of papillae were observed, with 11–21 papillae/

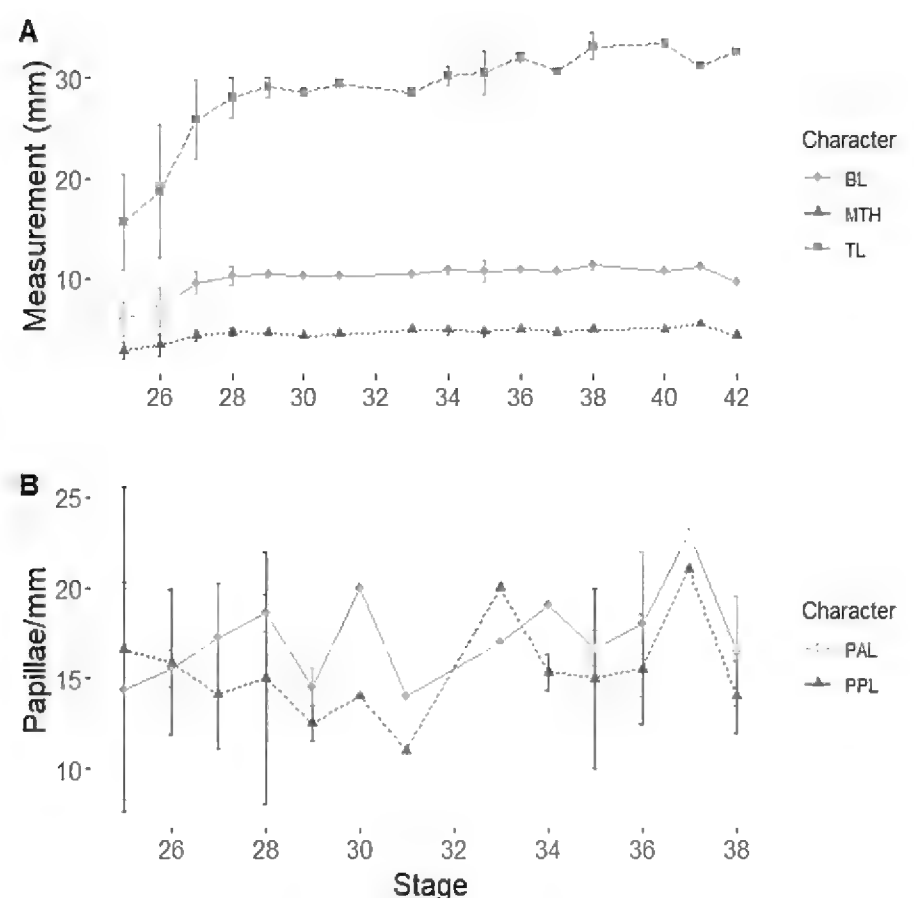


Fig. 3. Ontogenetic variation of body: some morphometric characters (a) and papillae per millimeter counts (b) through Gosner (1960) stages. BL = body length, MTH = maximum tail height, TL = tail length, PAL = number of papillae/mm on anterior lip, PPL = number of papillae/mm on posterior lip.

mm (Fig. 3b). Regarding the length of the rows of teeth on the posterior lip, they could either all have the same length ($P1 = P2 = P3$) or two equal and one shorter ($P1 = P2 > P3$ or $P2 > P1 = P3$). Metamorphics of stages 42 ($n = 1$; TL = 18.2 mm, TAL = 12.5 mm, BW = 4.5 mm), 43 ($n = 2$, TL = 17.7 mm, TAL = 9.0 mm, BW = 4.0 mm) and 45 ($n = 1$, TL = 11.4 mm, BW = 4.2) showed reductions in total length, maximum tail height, and tail length. In addition, other structures such as the oral disc, keratinized ornamentation (UPJ, LJS, and tooth rows), blowhole, and cloacal tube disappear.

Tadpoles are generally uniformly dark-backed, although some specimens have minute golden spots toward the front of the body. Free-swimming tadpoles have a gold stripe toward the back of the eyes, which have tiny gold dots (Fig. 4a–b). Between the eyes and the tip

of the snout, golden spots are directed laterally, passing under the nostrils. The back of the body is dark brown with golden spots (in some cases evenly brown). The tail in lateral view presents dark brown spots, encompassing the dorsal and ventral fins. The caudal musculature is greenish yellow. In ventral view, it is whitish to greyish, and slightly translucent. When preserved, the body is light brown in dorsal view, but completely black in some cases. The golden stripe and spots disappear, being replaced by brown or black spots on the tail. In the distal region, the tail becomes translucent. Ventrally, the intestine is visible under the skin, showing a dark brown coloration.

The free-swimming tadpoles of “*C.*” *ruthveni* ss generally inhabit pools in small mountain lotic systems that are located in the shade under large trees and surrounded by medium-sized vegetation. These sites present benthic and hidden habitats. In pools with a lot of sun exposure, individuals hide under rocks, marginal vegetation, or the bottom leaf litter, or are partially buried in the sand. The tadpoles have an apparently omnivorous diet, since some individuals were found feeding on leaves and flowers that fell into the ponds as well as earthworm remains and a dead conspecific (Fig. 4c–d).

Courtship and Reproductive Mode

The observations of four pairs involved in courtship in Las Tinajas and Pozo Azul were used to build a general scheme of courtship behavior that had been previously unknown (Fig. 5). It is worth mentioning that the courtship of “*C.*” *ruthveni* ss exhibits some slight variations among the partial observations made in this study. All the courtship events began with the issuance of the mating call by the male from various

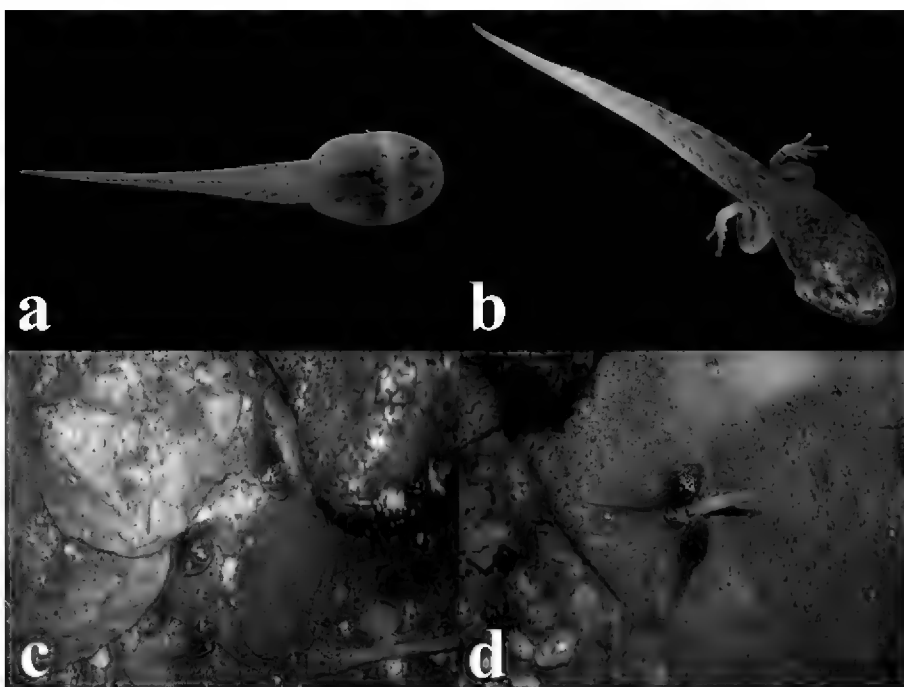


Fig. 4. Color in life of uncollected free-swimming tadpoles of “*Colostethus*” *ruthveni sensu stricto* in stage 28 (a) and 37 (b) from La Victoria; and records *in situ* of the consumption of plant material (c) and conspecific larvae (d). Photos by Andrés Camilo Montes-Correa (a), Juan Manuel Renjifo (b), and Fredy Polo-Córdoba (c, d).

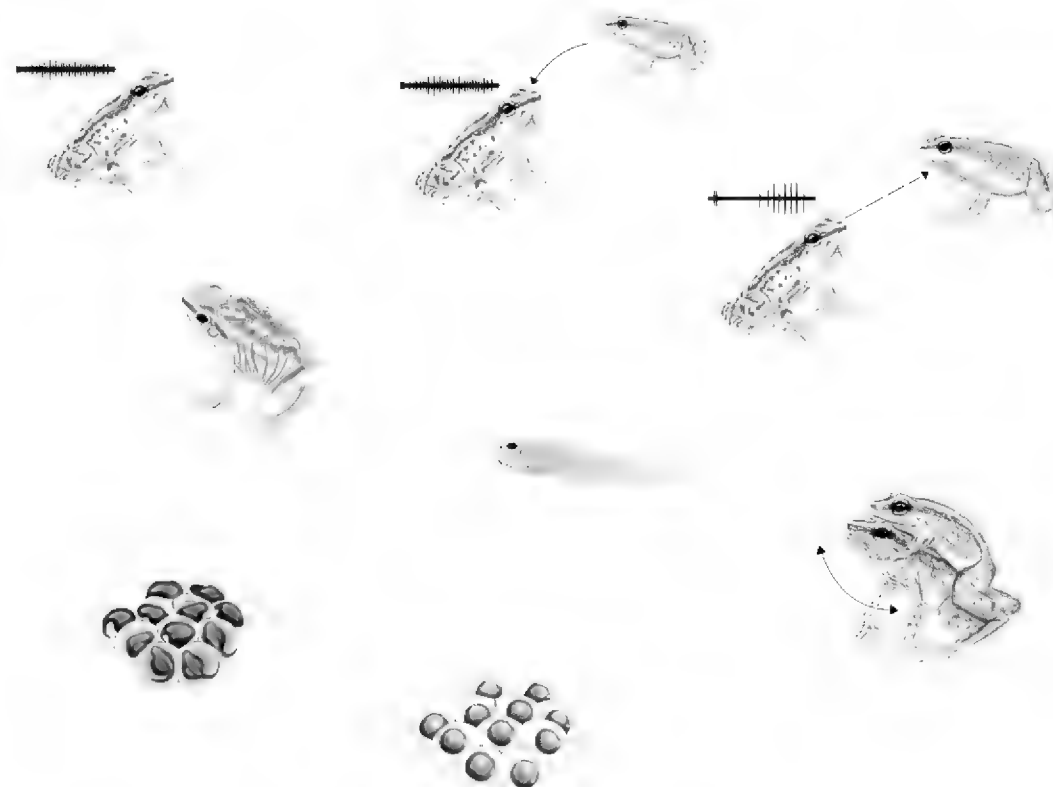


Fig. 5. Simplified schematic representation of courtship behavior and reproductive mode known up to now for “*Colostethus*” *ruthveni sensu stricto*, with the following steps: male issues advertisement call, female approaches, male issues courtship call, amplexus occurs from axilla to head, laying and development of terrestrial eggs, transport of larvae, and laying of free-swimming tadpoles in a pool or stream. Drawings by Jhuliyana Lopez-Caro.

substrates (e.g., cavities between the boulders, on fallen logs on the edge of the ravine, or on the broad leaves of grazing vegetation). The males darken in color during vocalization (color change). This results in positive phonotaxis by the females that approach the vocalization sites. In most of the observations, during the first phase of the close encounter between the male and the female, the male began to “circle,” circling the female with small jumps while she issued the mating call. Later, the male exhibited “body raising,” a raised posture stretching all four legs, followed by a “throat display” of inflating the vocal sac. In other cases, there were tactile interactions that included the stealthy approach of the male to the female, who then rubbed his forelimbs on her back, like a “massage.” This was followed by the emission of the courtship or advertisement call and cephalic amplexus, which always occurred in hidden spaces, such as the spaces between boulders or in cavities of fallen logs. On one occasion for a few seconds, pseudo positions or variants ranging from axillary to cephalic grasping were observed, ending in the latter. Only in one pair during cephalic amplexus was the male observed to make “toe trembling” behavior (see Supplementary Material 2: video).

The positions of nine egg masses were observed, five of which were deposited at the bases of the upper leaves (Fig. 6a) of low-growing plants hidden by the foliage. Four egg masses (spawn) were laid on leaves just above the water surface. The remaining spawn was on a plant 0.57 m horizontally from the water and 14 cm high. However, it is possible that this spawn was originally on the surface of the water, but probably moved away from it due to the shrinking volume of water in the creek. In

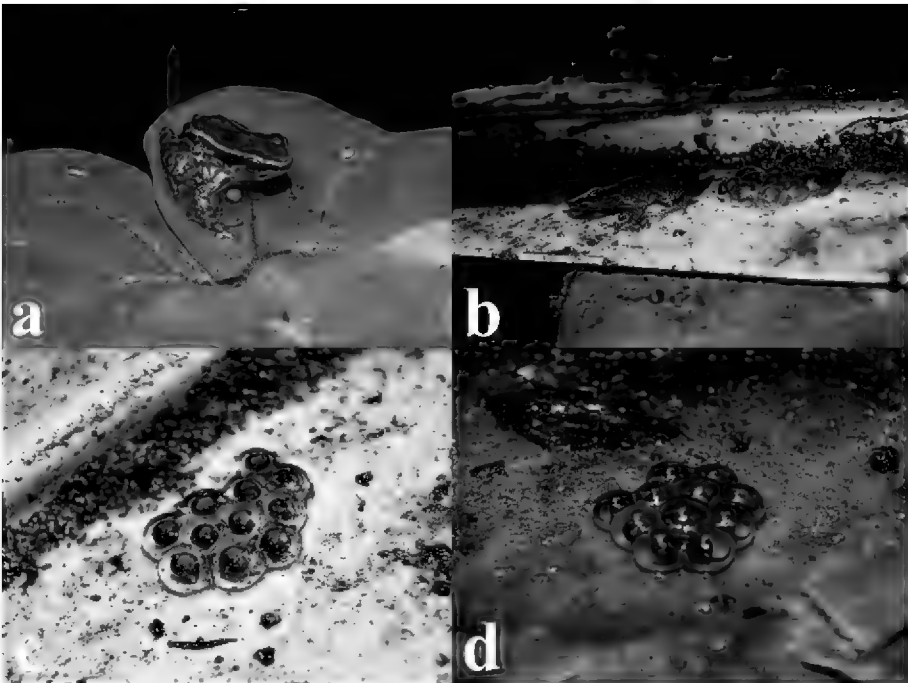


Fig. 6. Parental care and oviposition substrates of “*Colostethus*” *ruthveni sensu stricto*. Male attending to a posture on the upper side of a leaf (a) and under a plastic sheet (b). Detail of the posture on the plastic sheet (c) and leaf litter (d). *Photos by Liliana Saboyá-Acosta (a) and Fredy Polo-Córdoba (b, c, d).*

three of these positions, parental care by the males was detected. In addition, the use of artificial substrates was identified (Fig. 6b–c), as egg masses were deposited in a pile of damp cardboard and plastic sheets surrounded by leaf litter, both about 50 m from the nearest watercourse. Parental care was observed in both sexes. In addition, two egg masses were found at ground level. One of them was deposited in a hole in a rock covered by leaf litter, approximately two horizontal meters from the watercourse. The other was deposited directly on wet leaf litter (Fig. 6d) about four meters from the ravine. In neither of the two cases was parental care observed, but males were vocalizing around the spawn. Finally, in Sierra Minca a spawn was oviposited directly on the

Table 4. Summary of features of the nests of “*Colostethus*” *ruthveni sensu stricto*. Egg diameter is expressed as range (average ± standard deviation).

Site and month	Nest location	Nest height (cm)	Nest water distance (m)	Substrata	Number of eggs	Egg diameter (mm)	Parental care
Las Tinajas, Jun 2015							
1	Above the water	47	0	Leaf base	15	3.2–5.2 (4.2±0.6)	X
2	Above the water	31	0	Leaf base	15	2.5–4.5 (3.9±0.4)	X
3	Above the water	25	0	Leaf base	7	4.4–6.9 (5.3±0.8)	X
4	Above the water	20	0	Leaf base	2	2.7–3.9 (3.3±0.8)	
5	On the floor	14	0.57	Leaf base	6	1.8–2.8 (2.4±0.3)	
Bella Vista, Aug 2015							
6	On the floor	0	50	Wet cardboard and leaf-litter	11	4.1–5.8 (4.5±0.5)	X
7	On the floor	0	53	Plastic lamina and leaf-litter	11	4.2–6.0 (4.9±0.6)	X
Arimaca, Dec 2016							
8	On the floor	0	2	Rock hole and leaf-litter	11	3.5–7.3 (5.5±1.0)	
9	On the floor	0	4	Leaf-litter	10	4.1–5.8 (4.7±0.6)	
Sierra Minca, Jan 2021							
24	On the floor	0	3	Rock	3	3.7–4.1 (3.9±0.2)	X

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*

surface of a rock next to the creek flow. Although it was not possible for us to photograph this record, photographs taken by other researchers in the region that document it are available on social networks (https://www.instagram.com/p/BIBQrNqhLDI/?igshid=MzRlODBiNWFlZA%3D%3D&fbclid=IwAR1F3LUaS7Y9XIUhZOHQ6uqUbhK3cRYSkZ5FRjVNHbhncKp-m32OJ9n3y74&img_index=2). The data from the spawn of “*C.*” *ruthveni* ss reported in this study suggests that this species produces a small number of eggs (Table 4, range = 2–15 eggs; mean \pm standard deviation = 9.2 ± 4.4 eggs) that are small in size (1.8–7.3 mm, 4.4 ± 1).

Distribution of Larval and Reproductive Characters

New information was obtained for 12 of the 35 characters examined: 10 reproductive characters and two larval characters (Fig. 7, see Supplementary Material 1: Tables S2 and S3; and Supplementary Material 3). Under the hypothesis of the relationships of the Dendrobatoidea genera of Grant et al. (2017, Appendix 1), 27 characters

(17 larval, 10 reproductive) can be considered symplesiomorphic for the group “*C.*” *ruthveni*. The caudal coloration of the larvae is arranged in dispersed melanophores that are grouped in diffuse spots [93(0); a character number in Appendix 1 of Grant et al. (2017, state of character)], occurrence of reproductive amplexus [115(1)], and larval habitat in wells and streams [123(0)] can indicate reversions to ancestral states. Likewise, the presence of lateral line stitches [106(1), character examined by Grant et al. 2017] could be considered as an apomorphy. Two polymorphic characters were observed. In the case of the laying site, terrestrial clutches at ground level [118(1)] are a symplesiomorphic state, while terrestrial clutches above the ground or water level [118(3)] are an apomorphy. On the diet of exotrophic tadpoles, predatory habits [125(1)] consist of a symplesiomorphy, while detritivorous habits [125(2)] consist of a reversal.

Regarding the continuous character of A-2 gap/ODW (%), the ranges were so wide among most of the genera that it was not possible to classify them

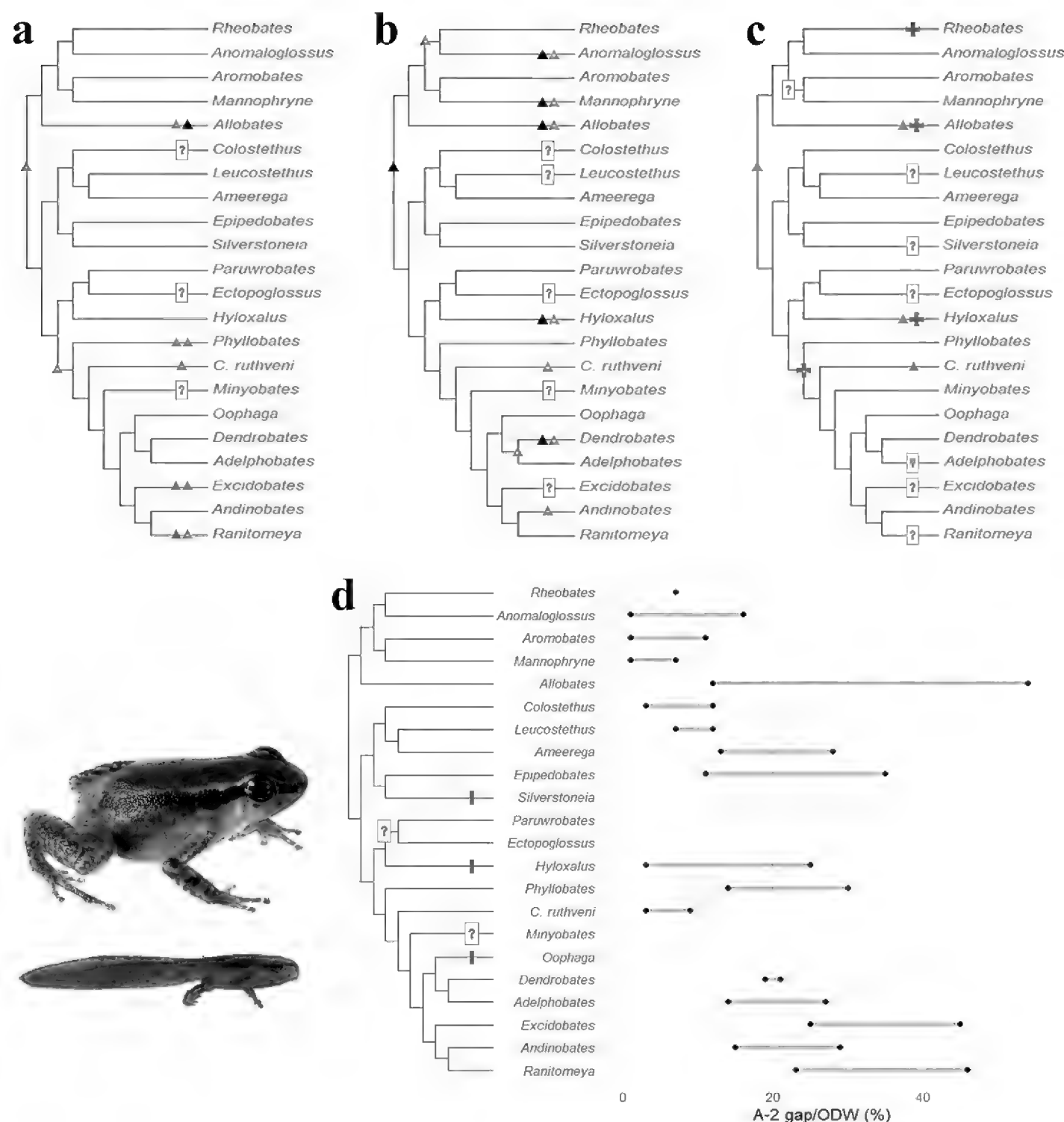


Fig. 7. Mapping of selected larval and reproductive character on the topology with summary of phylogenetic relationships of genera across Dendrobatoidea by Grant et al. 2017. 93. Larval caudal coloration (a): 0. vertically striped (▲); 1. scattered melanophores clumped to form diffuse blotches (▲); 2. evenly pigmented (▲). 106. Lateral line stitches (b): 0. absent (▲); 1. present (▲). 115. Reproductive amplexus position (c): 0. absent (▲); 1. present (▲); not applicable (+). Range of A-2 gap/oral disc ratio in percentage (d). Adult male and metamorphic tadpole (stage 45) of “*Colostethus*” *ruthveni* ss from Minca, Santa Marta, Magdalena. Photographs are not scaled.

in a single category (see Supplementary Material 1: Table S4). Only the “*C.*” *ruthveni* group, *Rheobates*, and *Mannophryne* showed strictly narrow A-2 gaps. Likewise, the genera *Anomalogluss*, *Aromobates*, *Colostethus*, and *Leucostethus* had narrow to moderate A-2 gaps (in all cases the A-2 gap/ODW $\leq 16\%$). Something similar occurred with *Hyloxalus*, where most of the species with the presence of an A-2 gap (absent in eight species), showed gaps falling between narrow and moderate (A-2 gap/ODW = 3–13%). The exception was *H. sauli*, which had a considerably wider A-2 gap (A-2 gap/ODW = 25%). The genera *Allobates*, *Amereega*, *Epipedobates*, *Phyllobates*, *Dendrobates*, *Adelphobates*, and *Andinobates* all had moderate to wide A-2 gaps. Only the genera *Excidobates* and *Ranitomeya* had strictly wide A-2 gaps. These findings indicated to us that “*C.*” *ruthveni* is the only Dendrobatinae with a narrow A-2 gap.

Discussion

Relative Abundance and Microhabitat Use

“*Colostethus*” *ruthveni* ss is an abundant species, at least during the periods and at the sites sampled in this study. Although these data do not allow us to assume that the relative abundance is constantly high, they suggest that the populations can remain very conspicuous, despite the marked water deficit in the lower range of the SNSM (< 1000 m asl) during the dry season (Fundación Pro-Sierra Nevada 1998). The variations of the relative abundance in each sampling event could be the product of multiple factors inherent to the biology of “*C.*” *ruthveni* ss, such as the occupancy and detection probabilities, as well as those related to the characteristics of the sampling, such as the capture effort and the accessibility of the microhabitats for the observer (MacKenzie et al. 2002). Regarding the latter, the localities where the greatest abundance was recorded (Sierra Minca, Central Córdoba, and Las Tinajas) have easily accessible ravines, gentler slopes, and longer walkable stretches, where observers would have fewer physical difficulties in finding specimens.

The apparently stable population status of “*C.*” *ruthveni* ss in the study area may be related to the fact that the northwestern sector of the SNSM is one of the best-preserved areas of the entire massif since it has had the least loss of natural vegetation cover in the past two decades (Granda-Rodríguez et al. 2020). Previous studies of the other endemic amphibians of the SNSM in the genera *Atelopus*, *Tachiramantis*, *Serranobatrachus*, *Geobatrachus*, and *Ikakogi* (Granda Rodríguez et al. 2012; Granda-Rodríguez et al. 2020; Martínez-Baños et al. 2011; Roach et al. 2021) have shown high relative abundances, despite the presence of chytridiomycosis in the region (Flechas et al. 2017). This contrasts with the general condition of the conservation status of amphibians in the North-Andean region, where multiple

population declines have been documented (Womack et al. 2022). Although measuring population attributes such as population density is necessary, we suggest calculating relative abundance as it is a cost-effective and comparable technique that provides at least one population attribute which can serve as an early warning in case of declines in amphibian populations. As in many parts of Latin America, population information has never been documented for many species, and currently, the population status of many amphibians in Colombia remains unknown (Young et al., 2001; IUCN SSC Amphibian Specialist Group, 2018).

Despite the apparent stability in the relative abundance of amphibians in the northwestern SNSM, multiple potential threats must be addressed. With the recent “Peace Process” in Colombia and the dismantling of some of the insurgent armed groups, areas that were previously protected indirectly by the Colombian armed conflict are now available for use. Due to the increase in tourism and agricultural activity in the area, demographic growth in the distributional area of “*C.*” *ruthveni* has recently accelerated (Carvajalino-Slaghekke 2015; Guardiola 2019). This leads to an increase in infrastructure and water demand, as well as environmental disturbances that, together with poor wastewater management, can synergistically affect the population status of this and other amphibian species in the area. Therefore, the implementation of environmental management measures and territorial planning in this region should be encouraged. Although, “*C.*” *ruthveni* is currently considered a Near Threatened species, the recent discovery of cryptic diversity within “*C.*” *ruthveni* (Grant et al. 2017; Jiménez-Bolaño et al. 2019) will generate substantial changes in the interpretation of the conservation status, because the area of distribution and threats will be fragmented according to the number of species that make up this group and how they are distributed throughout the SNSM.

The microhabitat use data obtained in this study show that the greatest activity of “*C.*” *ruthveni* occurs in the lowest strata of the forest, below approximately 50 cm. Like other dendrobatoids from northern Colombia, “*C.*” *ruthveni* finds shelter and food in microhabitats on the forest floor such as rocks and leaf litter (Blanco-Torres et al. 2013, 2014; Granda-Rodríguez et al. 2018; Posso-Peláez et al. 2017). In addition, all the processes involved in reproductive biology known up to now, such as singing, courtship, amplexus, laying site, and others, also occur at the ground level or in the lowest strata of the forest.

Free-swimming Tadpoles

The tadpoles of “*C.*” *ruthveni* have the typical morphology of the larvae that were in the genus *Colostethus sensu lato* (Anganoy-Criollo 2013; Coloma 1995), but are currently included in various genera of the families

Aromobatidae and Dendrobatidae (Colostethinae and Hyloxalinae). Several character states of external larval morphology, such as the UJS notch, sinister gut, and projecting nostril rim, are ancestral (Sánchez 2013), and symplesiomorphically shared with Aromobatidae, Colostethinae, Hyloxalinae, and *Phylllobates*. On the other hand, the narrow A-2 gap and the moderately sized nostrils could constitute putative synapomorphies of the “C.” group *ruthveni*, if they are verified to be also present in group members that are not yet described (*sensu* Grant et al. 2017; Jiménez-Bolaño et al. 2019).

The external morphology of the free-swimming tadpoles of “C.” *ruthveni* from La Victoria agrees with the character states described by Anganoy-Criollo (2013), Sánchez (2013), and Anganoy-Criollo and Cepeda-Quilindo (2017). However, part of the material examined and determined by Sánchez (2013) does not correspond to “C.” *ruthveni* ss. One batch of specimens (ICN 35773) came from the southwestern sector of the SNSM, where “C.” sp. *ruthveni*-like is distributed (Grant et al. 2017; Jiménez-Bolaño et al. 2019). In addition, the ICN 35779 batch of specimens came from the Serranía de Perijá, a region part of the Andean mountain range that is not connected to SNSM (Granda-Rodríguez et al. 2014; Jiménez-Bolaño et al. 2019; Meza-Joya et al. 2019). These tadpoles may correspond to two aromobatids from the western foothills of the Serranía de Perijá, i.e., *Rheobates palmatus* Werner, 1899 or *Allobates ignotus* Anganoy-Criollo, 2012 (Anganoy-Criollo 2012; Granda-Rodríguez et al. 2018; Moreno-Arias et al. 2009; Romero-Martínez and Lynch 2012).

The states of some tadpole characters of “C.” *ruthveni* determined in this study showed strong variation with respect to the data reported by Ruthven and Gaige (1915). The illustrations provided in that study of back-riding and free-swimming tadpoles show the A-2 gap to be very wide (i.e., back-riding tadpole: A-2 gap/ODW = 30%, free-swimming tadpole: A-2 gap/ODW = 25%, calculated qualitatively from Ruthven and Gaige (1915, Figs. 1 and 3)). Furthermore, referring to the same free-swimming tadpole with a 20 mm TL, they suggested that “the upper second row of teeth is not always interrupted.” Although it was not possible to determine the larval stage (according to the tadpoles illustrated by them), our larvae of similar size (Stage 26, $n = 13$, TL = 16.7–23.3 mm) have a dramatically reduced A-2 gap on average (A-2 gap/ODW = 5.1%), but it is never absent. One possibility is that the marked differences in the A-2 gap ratio of Ruthven and Gaige (1915) tadpoles with respect to the recent literature (Anganoy-Criollo and Cepeda-Quilindo 2017) is due to ontogenic variation; however, this variation would far exceed that detected in our material, since the highest A-2 gap/ODW ratio was <8% at stage 26.

Another contrasting detail of the Ruthven and Gaige (1915) illustrations is that they suggest the UJS notch is absent, a condition contrary to the reports of recent

studies (UJS notch present, UJS “W-shaped;” Sánchez 2013; Anganoy-Criollo and Cepeda-Quilindo 2017). The presence of the UJS notch is a character with little variation, and it is easily distinguishable in free-swimming tadpoles (from stage 25; Sánchez 2013; MA Anganoy-Criollo, pers. comm.). Thus, the A-2 gap/ODW ratio and the absence of the UJS notch in the free-swimming tadpole (20 mm TL) illustrated by Ruthven and Gaige (1915) could be considered as atypical character states considering the recent evidence.

Courtship and Reproductive Mode

Like other reproductive behavior traits, courtship is undoubtedly one of the most conspicuous and fascinating features of dendrobatoids (Wells 2007; Summers and Tumulty 2014). Thus, the partial observations on courtship in “C.” *ruthveni* are similar to those reported in other dendrobatoid species such as *Mannophryne trinitatis* Garman, 1888, *Mannophryne collaris* Boulenger, 1912, *Anomaloglossus stepheni* Martins, 1989, *Allobates marchesianus* Melin, 1941, *Allobates femoralis* Boulenger, 1884, *Allobates paleovarzensis* Lima, Caldwell, Biavati, and Montanarin, 2010, *Allobates velocicantus* Souza, Ferrão, Hanken, and Lima, 2020, *R. palmatus*, *Colostethus panamansis* Dunn, 1933, *Hyloxalus toachi* Coloma, 1995, *Dendrobates auratus* Girard, 1855, and *Dendrobates tinctorius* Cuvier, 1797, where behaviors such as color changes, body raising, circling, throat displays, and tactile interactions have been observed (Coloma and Quiguango-Ubillús 2008; Dole and Durant 1974; Duellman 1966; Hödl and Amézquita 2001; Juncá 1998; Juncá and Rodrigues 2006; Lima et al. 2010; Lüddecke 1976; Montanarin et al. 2011; Pašukonis and Rojas 2019; Rocha et al. 2018; Rojas and Pašukonis 2019; Wells 1978, 1980a,b). The tactile interactions of “C.” *ruthveni* are similar to those described in *A. stepheni*, *Allobates subfolionidificans* Lima, Sánchez, and Souza 2007, and *D. tinctorius*, where the male approaches the female and places his front legs on her back, perhaps a stimulant prior to oviposition (Juncá and Rodrigues 2006; Rojas and Pašukonis 2019; Souza et al. 2017).

Of all the possible courtship variations within the Dendrobatoidea clade, some behaviors contrast with our observations. For example, something very particular that differs notably from the behavior of “C.” *ruthveni* ss is the upright posture on the hind legs observed during courtship only in *M. collaris*, which the authors called a “toe-dance” (Dole and Durant 1974). Likewise, in aposematic species such as *Ameerega braccata* Steindachner, 1864, *Ameerega flavopicta* Lutz, 1925, and *Oophaga sylvatica* Funkhouser, 1956, courtship is accompanied by the display of conspicuous coloration on the hidden surfaces through visual cues involving the movement of the limbs (Costa et al. 2006; Forti et al. 2013; Summers 1992). “Toe-trembling” is a very

common visual signal in anurans (Sloggett and Zeilstra 2008). However, exhibiting this signal in “*C.*” *ruthveni* ss during amplexus differs from that reported in species such as *Oophaga histrionica* Berthold, 1845, *D. auratus*, and *D. tinctorius*, where it has been observed during courtship, foraging, and agonistic interactions (Rojas and Pašukonis 2019; Silverstone 1973; Wells 1978). All of this variation in the courtship behavioral displays raises two questions that could be addressed from an evolutionary perspective in future research: Does aposematism have a direct relationship with the use of visual, acoustic, or tactile displays? Does aposematism somehow favor greater diversity of displays during courtship? The answers to these questions could provide an interesting evolutionary context, considering that most aposematic groups have a cryptic ancestor, whereas so far, the “*C.*” *ruthveni* group is the only cryptic coloration lineage with an aposematic ancestor.

Cephalic amplexus is one of the most striking characteristics of dendrobatoid courtship. It is present in at least 22 species and is strongly associated with terrestrial habits, representing 6.5% of the known diversity of the clade (Carvajal-Castro et al. 2020; Frost 2023). This behavior is quite complex, and the establishment of its homology depends largely on the reproductive context, where certain pre-ovipositional variants are not part of the cephalic grasp in a strict sense (Castillo-Trenn and Coloma 2008). In accordance with the above observations, “*C.*” *ruthveni* ss exhibits various forms of grasping in the nuptial embrace, resulting in intermediate points between the axillary position and the final cephalic position. This is similar to observations in *A. flavopicta*, where the axillary amplexus was initially reported (Costa et al. 2006). However, later observations determined intraspecific variation in the position of the embrace, with intermediate positions between axillary and cephalic amplexus, with the latter being the predominant variation (Magrini et al. 2010). However, this differs from what was reported in *Hyloxalus azureiventris* (Kneller and Henle 1985) and some cryptic species that were part of the extensive group *Colostethus sensu lato*, such as *Allobates caeruleodactylus* Lima and Caldwell, 2001, and *A. subfolionidificans*, in which this type of amplexus has not been recorded (Lima et al. 2002; Quiguango-Ubillús and Coloma 2008; Souza et al. 2017).

Several authors have considered parental care and larval transport as reproductive mode traits (Crump 2015; Duellman and Trueb 1994; Haddad and Prado 2005; Wells 2007). However, in their redefinition of the reproductive mode, Nunes-De-Almeida et al. (2021) excluded parental care as it is a difficult characteristic to identify, except for those cases where care involves feeding and incubation. This is interesting when compared with our observations. Considering the proposal of Haddad and Prado (2005), the annotations for the transport of larvae made by Ruthven and Gaige (1915) complemented with the masses of eggs at ground

level found in “*C.*” *ruthveni* ss in localities of the SNSM coincide with Mode 20 (eggs that hatch into exotrophic tadpoles that are transported to the water by the adult), which agrees with that exhibited by *C. panamansis* and other dendrobatoids of the *Ameerega*, *Silverstoneia*, *Epipedobates*, *Hyloxalus*, and *Allobates* genera (Wells 1980b; Lima et al. 2010; Summers and Tumulty 2014; Crump 2015).

Regarding the spawn positions above the ground level found for “*C.*” *ruthveni* in the Las Tinajas, these are similar to previous observations in *Allobates brunneus* (Cope, 1887), *Ameerega bilinguis* (Jungfer, 1989), *Ameerega hahneli* (Boulenger, 1884), *Allobates carajas* Simões, Rojas, and Lima, 2019, *Leucostethus fraterdanieli* (Silverstone, 1971), and *A. subfolionidificans* (Beirne and Whitworth 2011; Brown et al. 2019; Lima et al. 2009; Rojas-Morales et al. 2021; Simões et al. 2019). However, *A. subfolionidificans* differs from the remaining species because it deposits its eggs on the undersides of leaves (Lima et al. 2007; Souza et al. 2017). Unlike the species mentioned, the transport of larvae by “*C.*” *ruthveni* in this area was not observed, so we have elaborated two hypotheses to explain this difference: a) the transport and deposition of larvae in the pond is done immediately after hatching, and/or b) there is a mode without the transport of larvae in which they hatch and fall directly into the water (Mode 24, Haddad and Prado 2005). The latter hypothesis and the location of egg masses of “*C.*” *ruthveni* at and above ground level suggest some degree of phenotypic plasticity in the reproductive mode, as has been shown in the species *Dendropsophus ebraccatus* Cope, 1874 (Hylidae), which alternates between aquatic and terrestrial locations (Touchon and Warkentin 2008). The “*C.*” *ruthveni* ss egg masses in vegetation above ground level, as well as those deposited at ground level, correspond to modes 22 and 32 (Nunes-De-Almeida et al. 2021). Curiously, there is no reproductive mode in the scheme of Nunes-De-Almeida et al. (2021) that includes clutches deposited on rocky substrates that derive from tadpoles (indirect development) from exotrophic nutrition and lotic habitats. So far, only rocks have been documented as the ovipositing substrates in salamanders that produce direct developing offspring (Mode 35). Therefore, we propose an additional reproductive mode that continues the list of Nunes-De-Almeida et al. (2021): *Mode “75”: Terrestrial non-froth eggs laid on rock. Offspring with indirect development, lecithotrophic nutrition, exotrophic, and without parental feeding. Known only for anurans.* This reproductive mode variation is probably the product of phenotypic plasticity in this species against environmental conditions, although this must be tested experimentally both *in situ* and *ex situ* (Barboza 2014).

Distribution of Larval and Reproductive Characters

The hypothesis regarding the phylogenetic relationships

of the group “*C.*” *ruthveni* by Grant et al. (2017) could be considered a surprising result, since phylogenetic logistic models indicate that the probability of gaining the ability to sequester alkaloids or going from a cryptic to aposematic phenotype is considerably higher than that of a reversion (Santos et al. 2014). In fact, a study recently determined that group “*C.*” *ruthveni* lacks the ability to sequester epibatidines, the most widely distributed alkaloid of dendrobatoid frogs (Waters et al. 2023). However, this hypothesis has the robustness conferred by the Goodman-Bremer values and the YBIRÁ procedure that tests the stability of nodes through the removal of clades or “wildcard” terminals (Grant and Kluge 2008; Machado 2015). While our objective is not evaluating the plausibility the phylogenetic relationships of group “*C.*” *ruthveni* proposed by Grant et al. (2017), according to the new evidence, we believe that the sampling and refinement of certain characters could provide greater clarity about their relationships. First, how does it affect the analysis that “*C.*” *ruthveni* does not present the only phenotypic synapomorphy of the subfamily Dendrobatinae [160(1); the ability to sequester lipophilic alkaloids present]? However, we detected that at least 27 characters (Supplementary Table 5) were declared as a mixture of neomorphic and transformational characters. This character set includes the three synapomorphies of the clade group “*C.*” *ruthveni* + Dendrobatini (characters 71, 72, and 156). Although the declarations of these characters undoubtedly follow a logical sequence (Serenio 2007), examining the appearance/loss and the transformation of the states in the same character is inappropriate because that would violate the precept of the independence of characters. Including these considerations and the additional characters presented here could at best reinforce the Grant et al. (2017) hypothesis, or perhaps provide a different perspective of the relationships of the group “*C.*” *ruthveni* and even Dendrobatoidea.

Considering these aspects of Grant et al. (2017) on the relationships of Dendrobatoidea, this is the most comprehensive and complete approach to the topic, and we will base our hypotheses on the evolution of larval and reproductive characters on this approach. In general, the larval morphology of Dendrobatoidea is conservative, where there is a generalized ancestral morphological pattern symplesiomorphically shared by Aromobatidae, Colostethinae, Hyloxalinae, and some members of Dendrobatinae such as *Phyllobates* and the group “*C.*” *ruthveni* (Sánchez 2013). Compiling the data obtained by Sánchez (2013), Grant et al. (2017), and this study, this ancestral body plan can be characterized by the caudal coloration of the tadpole with scattered melanophores clumped to form diffuse blotches [93(1)], the presence of a notch in the upper jaw sheath [103(0)], the cloacal tube in dextral position [104 (0)], the long gut concealing other organs [107 (0)], and the presence of a projection of the external sagittal edge of the nostril [108 (1)].

Although the larval characters of “*C.*” *ruthveni* mostly correspond to a compendium of symplesiomorphies, some provide an interesting perspective. For example, the caudal coloration in “*C.*” *ruthveni* as clusters of melanophores forming diffuse spots [93(1)] corresponds to the loss of an acquired novelty in the Dendrobatinae subfamily clade [93(2)]. Both this reversal and the presence of lateral line stitches [106 (1)] could constitute a synapomorphy of the “*C.*” *ruthveni* if they are shown to be present in the undescribed members of the complex. Likewise, the presence of lateral line stitches could constitute a synapomorphy of the clade group “*C.*” *ruthveni* + Dendrobatini, if the presence of this character state is demonstrated in *Minyobates steyermarki* (Rivero, 1971). The A-2gap/ODW ratio also proved to be valuable for the diagnosis of the genera, despite the strong intrageneric variation detected. Intergeneric variation in A-2 gap width in the phylogenetic context of Grant et al. (2017) suggests that narrow to moderate gaps could be the ancestral condition and that moderate to wide gaps evolved independently in *Allobates*, *Amereega*, *Epipedobates*, a species of *Hyloxalus*, and the subfamily Dendrobatinae. In that sense, the presence of a narrow A-2 gap in “*C.*” *ruthveni* could be a reversal, and a synapomorphy in the case that its presence is verified in the other members of the group.

Of the reproductive characters, the occurrence of amplexus [115(1)] and of cephalic grasping [116(1)] are quite informative. For decades, cephalic amplexus was a dendrobatoid synapomorphy with multiple subsequent losses (Myers et al. 1991). However, from total evidence-based phylogenies, Grant et al. (2006) noted that cephalic amplexus has arisen in three independent lineages within the clade (*Anomaloglossus beebei* Noble 1923, Colostethinae, and *M. steyermarki*). Nevertheless, the occurrence of this character is known to be much more widely distributed throughout the Dendrobatoidea, being present in some *Anomaloglossus*, *Allobates*, *Colostethinae*, *Paruwrobates*, *Hyloxalus* and the group “*C.*” *ruthveni* (Bourne et al. 2001; Carvajal-Castro et al. 2020; Castillo-Trenn and Coloma 2008; Forti et al. 2013; Grant and Castro 1998; Grant et al. 2017 [see Supplementary Material S4]; Jungfer 1989; Juncá 1998; Lima et al. 2010; Magrini et al. 2010; Montanarin et al. 2011; Myers and Burrowes 1987; Myers et al. 1978; Quiguango-Ubillús and Coloma 2008; Rocha et al. 2018; Roithmair 1994; Souza et al. 2020; Wells 1980c). So far, this is the only member of the subfamily Dendrobatinae that still retains some form of amplexus, as the observations of Myers (1987) on *M. steyermarki* may be related to aggressive behavior, i.e., possible male-fighting (López-López et al. 2016), and for this reason it must be verified (Castillo-Trenn and Coloma 2008). All the above observations suggest that the larval and reproductive characters are a good source of information for understanding the relationships between the genera of Dendrobatoidea, and also that some characteristics

such as free-swimming tadpoles with a lateral line and a narrow A-2 gap, as well as the occurrence of cephalic amplexus, are useful for diagnosing the group “C.” *ruthveni*.

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Literature Cited

- Almendáriz CA, Ron SR, Brito MJ. 2012. Una especie nueva de rana venenosa de altura del género *Excidobates* (Dendrobatoidea: Dendrobatidae) de la Cordillera del Cóndor. *Papeis Avulsos de Zoologia* 52(32): 387–399.
- Altig R, McDiarmid RW. 1999. Body plan, development, and morphology. Pp. 24–51 In: *Tadpoles. The Biology of Anuran Larvae*. Editors, McDiarmid RW, Altig R. University of Chicago Press, Chicago, Illinois, USA. 454 p.
- Anganoy-Criollo M. 2012. A new species of *Allobates* (Anura, Dendrobatidae) from the western flank of the Serranía de Perijá, Colombia. *Zootaxa* 3308(1): 49–62.
- Anganoy-Criollo M. 2013. Tadpoles of the High-Andean *Hyloxalus subpunctatus* (Anura: Dendrobatidae) with description of larval variation and species distinction by larval morphology. *Papeis Avulsos de Zoologia* 53(15): 211–224.
- Anganoy-Criollo M, Cepeda-Quilindo B. 2017. Redescription of the tadpoles of *Epipedobates narinensis* and *E. boulengeri* (Anura: Dendrobatidae). *Phyllomedusa* 16(2): 155–182.
- Barboza FR. 2014. Retomando el concepto de plasticidad fenotípica en el estudio de los modos reproductivos de anfibios anuros. *Boletín de la Sociedad Zoológica del Uruguay* 23(1): 1–14.
- Beirne C, Whitworth A. 2011. *Frogs of the Yachana Reserve*. Global Vision International, Exeter, United Kingdom. 109 p.
- Blanco-Torres A, Navarro-Gutiérrez K, Gutiérrez-Moreno LC. 2013. *Colostethus inguinalis* (Common Rocket Frog) diet. *Herpetological Review* 44(3): 493–494.
- Blanco-Torres A, Ordóñez-Pachón MP, Franco-Rozo MC. 2014. *Colostethus ruthveni* (Santa Marta poison arrow frog) diet. *Herpetological Review* 45(3): 476.
- Bourne GR, Collins AC, Holder AM, McCarthy CL. 2001. Vocal communication and reproductive behavior of the frog *Colostethus beebei* in Guyana. *Journal of Herpetology* 35(2): 272–281.
- Breder CM. 1946. Amphibians and reptiles of the Rio Chucunaque drainage, Darien, Panama, with notes on their life histories and habitats. *Bulletin of the American Museum of Natural History* 86(8): 375–346.
- Brown JL, Siu-Ting K, Von May R, Twomey E, Guillory WX, Deutsch MS, Chávez G. 2019. Systematics of the *Ameerega rubriventris* complex (Anura: Dendrobatidae) with descriptions of two new cryptic species from the East-Andean versant of Peru. *Zootaxa* 4712(2): 211–235.
- Brown JL, Twomey E, Morales V, Summers K. 2008. Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. *Behaviour* 145(9): 1,139–1,165.
- Caldwell JP. 2005. A new Amazonian species of *Cryptophyllobates* (Anura: Dendrobatidae). *Herpetologica* 61(4): 449–461.
- Caldwell JP, Myers CW. 1990. A new poison frog from Amazonian Brazil, with further revision of the *quinquevittatus* group of *Dendrobates*. *American Museum Novitates* 2988: 1–21.
- Caldwell JP, Lima AP. 2003. A new amazonian species of *Colostethus* (Anura: Dendrobatidae) with a nidicolous tadpole. *Herpetologica* 59(2): 219–234.
- Caldwell JP, Lima AP, Biavati GM. 2002. Descriptions of tadpoles of *Colostethus marchesianus* and *Colostethus caeruleodactylus* (Anura: Dendrobatidae) from their type localities. *Copeia* 2002(1): 166–172.
- Carvajal-Castro JD, López-Aguirre Y, Ospina-L AM, Santos JC, Rojas B, Vargas-Salinas F. 2020. Much more than a clasp: evolutionary patterns of amplexus diversity in anurans. *Biological Journal of the Linnean Society* 129(3): 652–663.
- Carvajalino-Slaghekke A. 2015. Territorio e identidad en la Sierra Nevada de Santa Marta (Colombia) Territory. *Revista Latina de Sociología* 8(3): 94–112.
- Castillo-Trenn P. 2004. Description of the tadpole of *Colostethus kingsburyi* (Anura: Dendrobatidae) from Ecuador. *Journal of Herpetology* 38(4): 600–606.
- Castillo-Trenn P, Coloma LA. 2008. Notes on behavior and reproduction in captive *Allobates kingsburyi* (Anura: Dendrobatidae), with comments on the evolution of reproductive amplexus. *International Zoo Yearbook* 42(1): 58–70.

- Coloma LA. 1995. Ecuadorian frogs of the genus *Colostethus* (Anura: Dendrobatidae). *The University of Kansas Natural History Museum Miscellaneous Publications* 87: 1–72.
- Costa RC, Facure KG, Giaretta AA. 2006. Courtship, vocalization, and tadpole description of *Epipedobates flavopictus* (Anura: Dendrobatidae) in southern Goiás, Brazil. *Biota Neotropica* 6(1): [no pagination].
- Crump ML. 2015. Anuran reproductive modes: evolving perspectives. *Journal of Herpetology* 49(1): 1–16.
- Crump ML, Scott NJJ. 1994. Visual encounter survey. Pp 84–91 In: *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians*. Editors, Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- Dias PHDS, Anganoy-Criollo M, Guayasamin JM, Grant T. 2018a. The tadpole of *Epipedobates darwinwallacei* Cisneros-Heredia and Yáñez-Muñoz, 2011 (Dendrobatidae: Colostethinae), with new synapomorphies for *Epipedobates*. *South American Journal of Herpetology* 13(1): 54–63.
- Dias PHDS, Brandão AP, Grant T. 2018b. The buccopharyngeal morphology of the tadpole of *Ameerega flavopicta* (Anura: Dendrobatidae: Colostethinae), with a redescription of its external morphology. *Herpetologica* 74(4): 323–328.
- Dias PHDS, Anganoy-Criollo M, Rada M, Grant T. 2021. The tadpoles of the funnel-mouthed dendrobatids (Anura: Dendrobatidae: Colostethinae: *Silverstoneia*): external morphology, musculoskeletal anatomy, buccopharyngeal cavity, and new synapomorphies. *Journal of Zoological Systematics and Evolutionary Research* 59(2): 691–717.
- Dole JW, Durant P. 1974. Courtship behavior in *Colostethus collaris* (Dendrobatidae). *Copeia* 1974(4): 988–990.
- Donnelly MA, Guyer C, de Sá RO. 1990. The tadpole of a dart-poison frog *Phylllobates lugubris* (Anura: Dendrobatidae). *Proceedings of the Biological Society of Washington* 103(2): 427–431.
- Duarte-Marín S, González-Acosta CC, Dias PHDS, Arias-Álvarez GA, Vargas-Salinas F. 2020. Advertisement call, tadpole morphology, and other natural history aspects of the threatened poison frog *Andinobates daleswansonii* (Dendrobatidae). *Journal of Natural History* 54(45–46): 3,005–3,030.
- Duellman WE. 1966. Aggressive behavior in dendrobatid frogs. *Herpetologica* 22(3): 217–221.
- Duellman WE. 2004. Frogs of the genus *Colostethus* (Anura: Dendrobatidae) in the Andes of Northern Peru. *Scientific Papers - Natural History Museum, The University of Kansas* 35: 1–49.
- Duellman WE, Trueb L. 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore, Maryland, USA. 670 p.
- Edwards SR. 1971. Taxonomic notes on South American *Colostethus* with descriptions of two new species (Amphibia, Dendrobatidae). *Proceedings of the Biological Society of Washington* 84(18): 147–162.
- Edwards SR. 1974. Taxonomic notes on South American dendrobatid frogs of the genus *Colostethus*. *Occasional Papers of the Museum of Natural History, The University of Kansas*. 30: 1–14.
- Flechas SV, Paz A, Crawford AJ, Sarmiento C, Acevedo A, Arboleda A, Bolívar-García W, Echeverry-Sandoval CL, Franco R, Mojica C, et al. 2017. Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity. *Biotropica* 49(5): 685–694.
- Forti LR, Mott T, Strüssmann C. 2013. Breeding biology of *Ameerega braccata* (Steindachner, 1864) (Anura: Dendrobatidae) in the Cerrado of Brazil. *Journal of Natural History* 47(35–36): 2,363–2,371.
- Fouquet A, Vacher JP, Courtois EA, Deschamps C, Ouboter P, Jairam R, Gaucher P, Dubois A, Kok PJR. 2019. A new species of *Anomaloglossus* (Anura: Aromobatidae) of the *stepheni* group with the redescription of *A. baeobatrachus* (Boistel and de Massary, 1999), and an amended definition of *A. leopardus* Ouboter and Jairam, 2012. *Zootaxa* 4576(3): 439–460.
- French CM, Burkette C, Reichle S, Brown JL. 2019. The tadpole of *Ameerega boehmei* in southeastern Bolivia. *Zootaxa* 4661(1): 197–200.
- Frost D. 2023. Amphibian species of the world: an online reference. Version 6.2. American Museum of Natural History, New York, USA. Available: <https://amphibiansoftheworld.amnh.org/index.php> [Accessed: 5 October 2023].
- Fundación Pro-Sierra Nevada. 1998. *Evaluación Ecológica Rápida. Definición de Áreas Críticas para la Conservación en la Sierra Nevada de Santa Marta, Colombia*. Editores, Fundación Pro-Sierra Nevada de Santa Marta, Ministerio del Ambiente - Unidad Administrativa Especial del Sistema de Parques Nacionales Naturales (UAESPNN), The Nature Conservancy, USAID, Embajada de Japón. Santa Marta, Magdalena, Colombia. 134 p.
- Funkhouser JW. 1956. New frogs from Ecuador and southwestern Colombia. *Zoologica: Scientific Contributions of the New York Zoological Society* 41(9): 73–80.
- González-Maya JF, González M, Zárrate-Charry D, Charry F, Cepeda AA, Balaguera-Reina SA. 2011. A new population record and conservation assessment of the Santa Marta Poison Arrow Frog *Colostethus ruthveni* Kaplan, 1997 (Anura: Dendrobatidae) from Sierra Nevada de Santa Marta, Colombia. *Journal of Threatened Taxa* 3(3): 1,633–1,636.
- Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3): 183–190.

- Granda-Rodríguez HD, Mozo A, Renjifo JM, Bolaños F. 2012 ¿Están declinando todos los *Atelopus* de alta montaña? El caso de las ranas arlequín de la Sierra Nevada de Santa Marta, Colombia. *Herpetotropicos* 7: 21–30.
- Granda-Rodríguez HD, Saboyá-Acosta LP, del Portillo-Mozo A, Renjifo JM. 2014. Range extension of dendrobatid frog *Colostethus ruthveni* Kaplan, 1997 (Anura: Dendrobatidae) in the Sierra Nevada de Santa Marta, Colombia. *Check List* 10(3): 674–676.
- Granda-Rodríguez HD, Montes-Correa AC, Jiménez-Bolaño JD, Anganoy-Criollo M. 2018. Natural history and conservation of the Nurse Frog of the Serranía del Perijá *Allobates ignotus* (Dendrobatoidea: Aromobatidae) in northeastern Colombia. *Acta Herpetologica* 13(1): 51–64.
- Granda-Rodríguez HD, Montes-Correa AC, Jiménez-Bolaño JD, Alaniz AJ, Cattán PE, Hernáez P. 2021. Insights into the natural history of the endemic Harlequin Toad, *Atelopus laetissimus* Ruiz-Carranza, Ardila-Robayo, and Hernández-Camacho, 1994 (Anura: Bufonidae), in the Sierra Nevada de Santa Marta, Colombia. *Amphibian & Reptile Conservation* 14(1) [General Section]: 29–42 (e221).
- Grant T. 1998. Una nueva especie de *Colostethus* del grupo *edwardsi* de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 22(84): 423–428.
- Grant T, Castro F. 1998. The cloud forest *Colostethus* (Anura, Dendrobatidae) of a region of the Cordillera Occidental of Colombia. *Journal of Herpetology* 32(3): 378–392.
- Grant T, Kluge AG. 2008. Clade support measures and their adequacy. *Cladistics* 24(6): 1,051–1,064.
- Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299: 1–262.
- Grant T, Acosta A, Rada M. 2007. A name for the species of *Allobates* (Anura: Dendrobatoidea: Aromobatidae) from the Magdalena Valley of Colombia. *Copeia* 2007(4): 844–854.
- Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV. 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology* 12(s1): S1–S90.
- Guardiola ADR. 2019. Potencial turístico de Santa Marta (Colombia): Una revisión de su desarrollo, crecimiento y barreras. *Espacios* 40(6): 22.
- Haddad CFB, Prado CPA. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* 55(3): 207–217.
- Hills RL, Kaylock JB, Gagliardo RW, Timpe EK, Griffith E, Ross H. 2011. Observations on the captive reproduction of *Colostethus pratti* (Boulenger, 1899). *Herpetological Review* 42(3): 365–367.
- Hödl W, Amézquita A. 2001. Visual signaling in anuran amphibians. Pp. 121–141 In: *Anuran Communication*. Editor, Ryan MJ. Smithsonian Institution Press, Washington, DC, USA. 252 p.
- Inger RF. 1994. Distribution of microhabitat. Pp. 60–66 In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Editors, Heyer RW, Donnelly MA, McDiarmid RW, Hayek LA, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- IUCN SSC Amphibian Specialist Group. 2018. *Colostethus ruthveni*. Available: <https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T55144A85891000.en> [Accessed: 5 May 2023].
- Jiménez-Bolaño JD, Montes-Correa AC, Polo-Córdoba F, Linares-Vargas K, Vergara-Gil D, Barrio-Amorós CL, Koch C. 2019. Acoustic repertory of the “*Colostethus*” *ruthveni* group (Anura: Dendrobatidae) and comments on their distribution in the Sierra Nevada de Santa Marta, Colombia. *Salamandra* 55(1): 27–38.
- Johnson DH. 1980. The comparison of usage and availability measurements for evaluating resource preference. *Ecology* 61(1): 65–71.
- Juncá FA. 1998. Reproductive biology of *Colostethus stepheni* and *Colostethus marchesianus* (Dendrobatidae), with the description of a new anuran mating behavior. *Herpetologica* 54(3): 377–387.
- Juncá FA, Rodrigues M. 2006. The reproductive success of *Colostethus stepheni* (Anura: Dendrobatidae). *Studies on Neotropical Fauna and Environment* 41(1): 9–17.
- Juncá FA, Altig R, Gascon C. 1994. Breeding biology of *Colostethus stepheni*, a dendrobatid frog with a nontransported nidicolous tadpole. *Copeia* 1994(3): 747–750.
- Jungfer K. 1989. Pfeilgiftfrösche der Gattung *Epipedobates* mit rot granuliertem Rücken aus dem Oriente von Ecuador und Peru. *Salamandra* 25(2): 81–98.
- Kaplan M. 1997. A new species of *Colostethus* from the Sierra Nevada de Santa Marta (Colombia) with comments on intergeneric relationships within the Dendrobatidae. *Journal of Herpetology* 31(3): 369–375.
- Klein B, Regnet RA, Krings M, Rödder D. 2020. Larval development and morphology of six Neotropical poison-dart frogs of the genus *Ranitomeya* (Anura: Dendrobatidae) based on captive-raised specimens. *Bonn Zoological Bulletin* 69(2): 191–223.
- Kok PJR, MacCulloch RD, Gaucher P, Poelman EH, Bourne GR, Lathrop A, Lenglet GL. 2006a. A new species of *Colostethus* (Anura, Dendrobatidae) from French Guiana with a redescription of *Colostethus beebei* (Noble, 1923) from its type locality.

- Phyllomedusa* 5(1): 43–66.
- Kok PJR, Sambhu H, Roopsind I, Lenglet GL, Bourne GR. 2006b. A new species of *Colostethus* (Anura: Dendrobatidae) with maternal care from Kaieteur National Park, Guyana. *Zootaxa* 1238(1): 35–61.
- Kok PJR, MacCulloch RD, Lathrop A, Willaert B, Bossuyt F. 2010. A new species of *Anomaloglossus* (Anura: Aromobatidae) from the Pakaraima Mountains of Guyana. *Zootaxa* 2660(1): 18–32.
- Kok PJR, Willaert B, Means DB. 2013. A new diagnosis and description of *Anomaloglossus roraima* (La Marca, 1998) (Anura: Aromobatidae: Anomaloglossinae), with description of its tadpole and call. *South American Journal of Herpetology* 8(1): 29–45.
- Krausman PR. 1999. Some basic principles of habitat use. Grazing behavior of livestock and wildlife. *Idaho Forest, Wildlife and Range Experiment Station Bulletin* 70: 85–90.
- La Marca E. 1985. A new species of *Colostethus* (Anura: Dendrobatidae) from the Cordillera de Merida, Northern Andes, South America. *Occasional Papers of the Museum of Zoology, University of Michigan* 710: 1–10.
- La Marca E. 1989. A new species of collared frog (Anura: Dendrobatidae: *Colostethus*) from Serrania de Portuguesa, Andes of Estado Lara, Venezuela. *Amphibia-Reptilia* 10(2): 175–183.
- La Marca E. 1993. Phylogenetic relationships and taxonomy of *Colostethus mandelorum* (Anura: Dendrobatidae), with notes on coloration, natural history, and description of the tadpole. *Bulletin of the Maryland Herpetological Society* 29(1): 4–19.
- La Marca E. 1994. Taxonomy of the frogs of the genus *Mannophryne* (Amphibia; Anura; Dendrobatidae). *Publicaciones de la Asociación de Amigos de Doñana* 4: 1–75.
- La Marca E, Mijares-Urrutia A. 1988. Description of the tadpole of *Colostethus mayorgai* (Anura: Dendrobatidae) with preliminary data on reproductive biology of the species. *Bulletin of the Maryland Herpetological Society* 24(3): 47–57.
- Lehtinen RM, Halley A. 2008. A description of the tadpole of *Mannophryne olmonae* (Anura: Aromobatidae). *Caribbean Journal of Science* 44(2): 260–264.
- Lescure J. 1976. Étude de deux têtards de *Phyllobates* (Dendrobatidae): *P. femoralis* (Boulenger) et *P. pictus* (Bibron). *Bulletin de la Société Zoologique de France* 101(2): 299–306.
- Lima AP, Caldwell JP. 2001. A new Amazonian species of *Colostethus* with sky blue digits. *Herpetologica* 57(2): 133–138.
- Lima AP, Keller C. 2003. Reproductive characteristics of *Colostethus marchesianus* from its type locality in Amazonas, Brazil. *Journal of Herpetology*. 37(4): 754–757.
- Lima AP, Caldwell JP, Biavati GM. 2002. Territorial and reproductive behavior of an Amazonian dendrobatid frog, *Colostethus caeruleodactylus*. *Copeia* 2002(1): 44–51.
- Lima AP, Sanchez DEA, Souza JRD. 2007. A new Amazonian species of the frog genus *Colostethus* (Dendrobatidae) that lays its eggs on the underside of leaves. *Copeia* 2007(1): 114–122.
- Lima AP, Caldwell JP, Biavati G, Montanarin A. 2010. A new species of *Allobates* (Anura: Aromobatidae) from Paleovárzea Forest in Amazonas, Brazil. *Zootaxa* 2337(1): 1–17.
- Lima AP, Caldwell JP, Strussmann C. 2009. Redescription of *Allobates brunneus* (Cope) 1887 (Anura: Aromobatidae: Allobatinae), with a description of the tadpole, call, and reproductive behavior. *Zootaxa* 1988(1): 1–16.
- Lima AP, Simões PI, Kaefer IL. 2014. A new species of *Allobates* (Anura: Aromobatidae) from the Tapajós River basin, Pará State, Brazil. *Zootaxa* 3889(3): 355–387.
- Lima AP, Simões PI, Kaefer IL. 2015. A new species of *Allobates* (Anura: Aromobatidae) from Parque Nacional da Amazônia, Pará State, Brazil. *Zootaxa* 3980(4): 501–525.
- Lips K. 1999. Mass mortality and population declines of anurans at an upland site in Western Panama. *Conservation Biology* 13(1): 117–125.
- López-López FJ, Rada M, Kahn TR. 2016. La Planada Poison Frog *Ameregea andina* (Myers and Burrowes 1987). Pp. 116–120 In: *A. Aposematic Poison Frogs (Dendrobatidae) of the Andean Countries: Bolivia, Colombia, Perú, and Venezuela. Tropical Fields Guides Series*. Editors, Kahn TR, La Marca E, Lötters S, Brown JL, Twomey E, Amézquita A. Conservation International, Arlington, Virginia, USA. 588 p.
- Lüddecke H. 1976. Einige Ergebnisse aus Feldbeobachtungen an *Phyllobates palmatus* (Amphibia, Ranidae) in Kolumbien. *Mitteilungen aus dem Institut Colombo Alemán de Investigaciones Científicas* 8: 157–163.
- Lüddecke H. 1999. Behavioral aspects of the reproductive biology of the Andean frog *Colostethus palmatus* (Amphibia: Dendrobatidae). *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 23(Suplemento Especial): 303–316.
- Lynch JD. 1982. Two new species of poison-dart frogs (*Colostethus*) from Colombia. *Herpetologica* 38(3): 366–374.
- Machado DJ. 2015. YBYRÁ facilitates comparison of large phylogenetic trees. *BMC Bioinformatics* 16(204): 2–5.
- MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA, Langtimm CA. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83(8): 2,248–2,255.
- Magrini L, Facure KG, Giaretta AA, da Silva WR, Costa RC. 2010. Geographic call variation and further notes on habitat of *Ameerega flavopicta* (Anura,

- Dendrobatidae). *Studies on Neotropical Fauna and Environment* 45(2): 89–94.
- Manjarrés-García G, Manjarrés-Pinzón G. 2004. Contribución al conocimiento hidrobiológico de la parte baja de los ríos de la vertiente noroccidental de la Sierra Nevada de Santa Marta, Colombia. *Revista Intropica* 1: 39–50.
- Marín CM, Molina-Zuluaga C, Restrepo A, Cano E, Daza JM. 2018. A new species of *Leucostethus* (Anura: Dendrobatidae) from the eastern versant of the Central Cordillera of Colombia and the phylogenetic status of *Colostethus fraterdanieli*. *Zootaxa* 4461: 359–380.
- Martínez-Baños V, Pacheco V, Ramírez-Pinilla MP. 2011. Abundancia relativa y uso de microhábitat de la rana *Geobatrachus walkeri* (Anura: Strabomantidae) en dos hábitats en Sierra Nevada de Santa Marta, Colombia. *Revista de Biología Tropical* 59(2): 907–920.
- Masche S, Zimmermann H, Pröhl H. 2010. Description and ecological observations of the tadpole of *Ranitomeya variabilis* (Anura: Dendrobatidae). *South American Journal of Herpetology* 5(3): 207–211.
- Menin M, Pinto RMC, Pegorini RJ, Da Silva MR. 2017. Redescription of the tadpole of *Ameerega hahneli* (Boulenger, 1884) (Anura: Dendrobatidae) with notes on ontogenetic variations and development habitats. *South American Journal of Herpetology* 12(3): 236–243.
- Meza-Joya FL, Chinchilla-Lemus W, Ramos E, Armesto O, Acevedo AA. 2019. New and noteworthy locality records of anurans from northeastern Andes of Colombia. *Herpetology Notes* 12: 61–69.
- Mijares-Urrutia A. 1991. Descripción del renacuajo de *Colostethus leopardalis* Rivero con algunos comentarios sobre su historia natural. *Amphibia-Reptilia* 12: 145–152.
- Mijares-Urrutia A, La Marca E. 1997. Tadpoles of the genus *Nephelobates* La Marca 1994 (Amphibia, Anura, Dendrobatidae), from Venezuela. *Tropical Zoology* 10(1): 133–142.
- Montanarin A, Kaefer IL, Lima AP. 2011. Courtship and mating behavior of the Brilliant-thighed Frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology, Ecology, & Evolution* 23(2): 141–150.
- Moraes LJCL, Pavan D, Lima AP. 2019. A new nurse frog of *Allobates masniger-nidicola* complex (Anura, Aromobatidae) from the east bank of Tapajós River, eastern Amazonia. *Zootaxa* 4648(3): 401–434.
- Moreno-Arias RA, Medina-Rangel GF, Carvajal-Cogollo JE, Castaño-Mora OV. 2009. Herpetofauna de La Serranía de Perijá. Pp. 449–470 In: *Colombia Diversidad Biológica VIII: La Alta Montaña de la Serranía del Perijá, Colombia*. Editor, Rangel-Ch JO. Instituto de Ciencias Naturales-Universidad Nacional de Colombia – CORPOCESAR, Bogotá DC, Colombia. 708 p.
- Myers CW. 1987. New generic names for some neotropical poison frogs (Dendrobatidae). *Papeís Avulsos de Zoologia* 36(25): 301–306.
- Myers CW, Burrowes PA. 1987. A new poison frog (*Dendrobates*) from Andean Colombia, with notes on a lowland relative. *American Museum Novitates* 2899: 1–17.
- Myers CW, Donnelly MA. 1997. A tepui herpetofauna on a granitic mountain (Tamacuari) in the borderland between Venezuela and Brazil: report from the Phipps Tapirapecó Expedition. *American Museum Novitates* 3213: 1–71.
- Myers CW, Donnelly MA. 2001. Herpetofauna of the Yutajé-Corocoro massif, Venezuela. Second report from the Robert G. Goelt American Museum–Terramar Expedition to the northwestern Tepuis. *Bulletin of the American Museum of Natural History* 261: 1–85.
- Myers CW, Daly JW, Malkin B. 1978. A dangerously toxic new frog (*Phylllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. *Bulletin of the American Museum of Natural History* 161(2): 307–366.
- Myers CW, Daly JW, Martínez V. 1984. An arboreal poison frog (*Dendrobates*) from Western Panama. *American Museum Novitates* 2783: 1–20.
- Myers CW, Paolillo OA, Daly JW. 1991. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of a new genus and species from the Venezuelan Andes. *American Museum Novitates* 3002: 1–33.
- Nunes-De-Almeida CHL, Haddad CFB, Toledo LF. 2021. A revised classification of the amphibian reproductive modes. *Salamandra* 57(3): 413–427.
- Páez-Vacas MI, Coloma LA, Santos JC. 2010. Systematics of the *Hyloxalus bocagei* complex (Anura: Dendrobatidae), description of two new cryptic species, and recognition of *H. maculosus*. *Zootaxa* 2711(1): 1–75.
- Pérez-Peña PE, Chavez G, Twomey E, Brown JL. 2010. Two new species of *Ranitomeya* (Anura: Dendrobatidae) from eastern Amazonian Peru. *Zootaxa* 2439(1): 1–23.
- Pezzuti TL, Araújo RB, Sanches PR, Santos FP, Dias-Souza MR, Costa-Campos CE. 2022. The tadpole of the endemic poison frog *Ameerega pulchripicta* (Silverstone, 1976) with the description of its chondrocranium (Anura: Dendrobatidae: Colostethinae). *Zootaxa* 5115(2): 295–300.
- Pisso-Florez GA, Liévano-Bonilla AC, Mazepa G, Mulder KP, Ramírez-Chaves HE. 2023. Distribution update, advertisement call, and phylogenetic position of the Malvasá Rocket Frog, *Hyloxalus pinguis* (Anura: Dendrobatidae). *South American Journal of Herpetology* 28(1): 78–88.
- Poelman EH, Verkade JC, van Wijngaarden RPA, Félix-

- Novoa C. 2010. Descriptions of the tadpoles of two poison frogs, *Ameerega parvula* and *Ameerega bilinguis* (Anura: Dendrobatidae) from Ecuador. *Journal of Herpetology* 44(3): 409–417.
- Portik DM, Streicher JW, Wiens JJ. 2023. Frog phylogeny: a time-calibrated, species-level tree based on hundreds of loci and 5,242 species. *Molecular Phylogenetics and Evolution* 188: 107907.
- Posso-Peláez C, Blanco-Torres A, Gutierrez-Moreno LC. 2017. Uso de microhábitats, actividad diaria y dieta de *Dendrobates truncatus* (Cope, 1861) (Anura: Dendrobatidae) en bosque seco tropical del norte de Colombia. *Acta Zoológica Mexicana* (Nueva Serie) 33(3): 490–502.
- Quiguango-Ubillús A, Coloma LA. 2008. Notes on behavior, communication, and reproduction in captive *Hyloxalus toachi* (Anura: Dendrobatidae), an endangered Ecuadorian frog. *International Zoo Yearbook* 42: 78–89.
- Regnet RA, Lukas P, Rödder D, Wipfler B, Solé M. 2023. Tadpole morphological characterization of *Ranitomeya variabilis* (Zimmermann and Zimmermann, 1988) (Anura: Dendrobatidae), with skeleton, muscle system and inner organs. *Zoomorphology* 142: 477–486.
- Rivero JA. 1971. Un nuevo e interesante *Dendrobates* (Amphibia, Salientia) del Cerro Yapacana de Venezuela. *Kasmera* 3: 389–396.
- Roach NS, Acosta D, Lacher TE. 2021. Shade coffee and amphibian conservation, a sustainable way forward? Understanding the perceptions and management strategies of coffee growers in Colombia. *Ecology & Society* 26(2): 33.
- Rocha SMC, Lima AP, Kaefer IL. 2018. Reproductive behavior of the amazonian nurse-frog *Allobates paleovarzensis* (Dendrobatoidea, Aromobatidae). *South American Journal of Herpetology* 13(3): 260–270.
- Rodriguez L, Myers CW. 1993. A new poison frog from Manu National Park, southeastern Peru (Dendrobatidae, *Epipedobates*). *American Museum Novitates* 3068: 1–16.
- Roithmair ME. 1994. Field studies of reproductive behavior in two dart-poison frog species (*Epipedobates femoralis*, *Epipedobates trivittatus*) in Amazonian Peru. *Herpetological Journal* 4(3): 77–85.
- Rojas B, Pašukonis A. 2019. From habitat use to social behavior: natural history of a voiceless poison frog, *Dendrobates tinctorius*. *PeerJ* 7(1) 1–30 (e7648).
- Rojas-Morales JA, La Marca E, Ramírez-Chaves HE. 2021. Advertisement call of a population of the nurse frog *Leucostethus fraterdanieli* (Anura: Dendrobatidae), with notes on its natural history. *Biota Colombiana* 22(1): 122–132.
- Romero-Martínez HJ, Lynch JD. 2012. Anfíbios de la Región Caribe. Pp. 677–701 In: *Colombia Diversidad Biológica XII. La Región Caribe de Colombia*. Editor, Rangel-Ch JO. Instituto de Ciencias Naturales-Universidad Nacional de Colombia, Bogotá DC, Colombia. 1,018 p.
- Ruiz-Carranza P, Ramírez-Pinilla MP. 1992. Una nueva especie de *Minyobates* (Anura: Dendrobatidae) de Colombia. *Lozania* 61: 1–16.
- Ruthven AG, Gaige HT. 1915. The breeding habitats of *Prostherapis subpunctatus* Cope. *Occasional Papers of the Museum of Zoology – University of Michigan* 11: 1–7.
- Sánchez DA. 2010. Larval development and synapomorphies for species groups of *Hyloscirtus* Peters, 1882 (Anura: Hylidae: Cophomantini). *Copeia* 2010(3): 351–363.
- Sánchez DA. 2013. Larval morphology of dart-poison frogs (Anura: Dendrobatoidea: Aromobatidae and Dendrobatidae). *Zootaxa* 3637(5): 569–591.
- Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLoS Biology* 7(3): 448–461 (e1000056).
- Santos JC, Baquero M, Barrio-Amorós C, Coloma LA, Erdtmann LK, Lima AP, Cannatella DC. 2014. Aposematism increases acoustic diversification and speciation in poison frogs. *Proceedings of the Royal Society B: Biological Sciences* 281(1796): 1–9.
- Savage JM. 1968. The Dendrobatid frogs of Central America. *Copeia* 1968(4): 745–776.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 954 p.
- Schulte R. 1990. Redescubrimiento y redefinición de *Dendrobates mysteriosus* (Myers 1982) de la Cordillera del Cóndor. *Boletín de Lima* 70: 57–68.
- Schulze A, Jansen M, Köhler G. 2015. Tadpole diversity of Bolivia’s lowland anuran communities: molecular identification, morphological characterization, and ecological assignment. *Zootaxa* 4016(1): 1–111.
- Sereno PC. 2007. Logical basis for morphological characters in phylogenetics. *Cladistics* 23(6): 565–587.
- Serrano-Rojas SJ, Whitworth A, Villacampa J, von May R, Gutiérrez RC, Padial JM, Chaparro JC. 2017. A new species of poison-dart frog (Anura: Dendrobatidae) from Manu Province, Amazon region of southeastern Peru, with notes on its natural history, bioacoustics, phylogenetics, and recommended conservation status. *Zootaxa* 4221(1): 71–94.
- Silverstone PA. 1971. Status of certain frogs of the genus *Colostethus*, with descriptions of new species. *Contributions in Science. Natural History Museum of Los Angeles County* 215: 1–8.
- Silverstone PA. 1973. Observations on the behavior and ecology of a Colombian poison-arrow frog, the Kokoe-Pa (*Dendrobates histrionicus* Berthold). *Herpetologica* 29(4): 295–301.
- Silverstone PA. 1975. Two new species of *Colostethus*

- (Amphibia: Anura: Dendrobatidae) from Colombia. *Natural History Museum of Los Angeles County – Contributions in Science* 268: 1–10.
- Simões PI, Lima AP, Farias IP. 2010. The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae). *Zootaxa* 2406(1): 1–28.
- Simões PI, Lima AP. 2012. The tadpole of *Allobates sumtuosus* (Morales, “2000” 2002) (Anura: Aromobatidae) from its type locality at Reserva Biológica do Rio Trombetas, Pará, Brazil. *Zootaxa* 3499(1): 86–88.
- Simões PI, Sturaro MJ, Peloso PLV, Lima AP. 2013. A new diminutive species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura, Aromobatidae) from the northwestern Rio Madeira-Rio Tapajós interfluvium, Amazonas, Brazil. *Zootaxa* 3609(3): 251–273.
- Sloggett JJ, Zeilstra I. 2008. Waving or tapping? Vibrational stimuli and the general function of toe twitching in frogs and toads (Amphibia: Anura). *Animal Behaviour* 76:1–4.
- Simões PI, Rojas D, Lima AP. 2019. A name for the nurse-frog (*Allobates*, Aromobatidae) of Floresta Nacional de Carajás, Eastern Brazilian Amazonia. *Zootaxa* 4550(1): 71–100.
- Souza JRD, Ferrão M, Hanken J, Lima AP. 2020. A new nurse frog (Anura: *Allobates*) from Brazilian Amazonia with a remarkably fast multi-noted advertisement call. *PeerJ* 8(2): 1–31.
- Souza JRD, Kaefer IL, Lima AP. 2017. The peculiar breeding biology of the Amazonian frog *Allobates subfolionidificans* (Aromobatidae). *Anais da Academia Brasileira de Ciências* 89(2): 885–893.
- Suarez-Mayorga AM, Lynch JD. 2001. Redescription of the tadpole of *Hyla vigilans* (Anura: Hylidae) and notes about possible taxonomic relationships. *Caribbean Journal of Science* 37(1–2): 116–119.
- Summers K. 1992. Mating strategies in two species of dart-poison frogs: a comparative study. *Animal Behaviour* 43: 907–919.
- Summers K, Tumulty J. 2014. Parental care, sexual selection, and mating systems in neotropical poison frogs. Pp. 289–320 In: *Sexual Selection: Perspectives and Models from the Neotropics*. Editors, Macedo RH, Machado G. Academic Press, Cambridge, Massachusetts, USA. 466 p.
- Touchon JC, Warkentin KM. 2008. Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. *Proceedings of the National Academy of Sciences of the United States of America* 105(21): 7495–7499.
- Twomey E, Brown JL. 2008. Spotted poison frogs: rediscovery of a lost species and a new genus (Anura: Dendrobatidae) from Northwestern Peru. *Herpetologica* 64(1): 121–137.
- Twomey E, Brown JL. 2009. Another new species of *Ranitomeya* (Anura: Dendrobatidae) from Amazonian Colombia. *Zootaxa* 2302(1): 48–60.
- van Wijngaarden R, Bolaños F. 1992. Parental care in *Dendrobates granuliferus* (Anura: Dendrobatidae), with a description of the tadpole. *Journal of Herpetology* 26(1): 102–105.
- Vargas-Salinas F, Castro-Herrera F. 1999. Distribución y preferencias de microhábitat en anuros (Amphibia) en bosque maduro y áreas perturbadas en Anchicayá, Pacífico Colombiano. *Caldasia* 21(1): 95–109.
- Vitt LJ, Caldwell JP. 2014. *Herpetology: An Introductory Biology of Amphibians and Reptiles*. Fourth Edition. Academic Press, London, United Kingdom. 776 p.
- von May R, Medina-Müller M, Donnelly MA, Summers K. 2008. The tadpole of the bamboo-breeding poison frog *Ranitomeya biolat* (Anura: Dendrobatidae). *Zootaxa* 1857(1): 66–68.
- Waters KR, Dugas MB, Grant T, Saporito RA. 2023. The ability to sequester the alkaloid epibatidine is widespread among dendrobatid poison frogs. *Evolutionary Ecology* 2023: 10260.
- Wells KD. 1978. Courtship and parental behavior in a Panamanian poison-arrow frog (*Dendrobates auratus*). *Herpetologica* 34(2): 148–155.
- Wells KD. 1980a. Behavioral ecology and social organization of a dendrobatid frog (*Colostethus inguinalis*). *Behavioral Ecology and Sociobiology* 6: 199–209.
- Wells KD. 1980b. Evidence for growth of tadpoles during parental transport in *Colostethus inguinalis*. *Journal of Herpetology* 14(4): 428–430.
- Wells KD. 1980c. Social behavior and communication of a dendrobatid frog (*Colostethus trinitatis*). *Herpetologica* 36(2): 189–199.
- Wells KD. 2007. *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago, Illinois, USA. 1,400 p.
- Weygoldt P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 25: 51–67.
- Womack MC, Steigerwald E, Blackburn DC, Cannatella DC, Catenazzi A, Che J, Koo MS, McGuire JA, Ron SR, Tarvin RD, et al. 2022. State of the Amphibia 2020: a review of five years of amphibian research and existing resources. *Ichthyology and Herpetology* 110(4): 638–661.
- Young BE, Lips KR, Reaser JK, Ibáñez R, Salas AW, Rogelio Cedeño J, Coloma LA, Ron S, La Marca E, Meyer JR, et al. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15: 1,213–1,223.

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*



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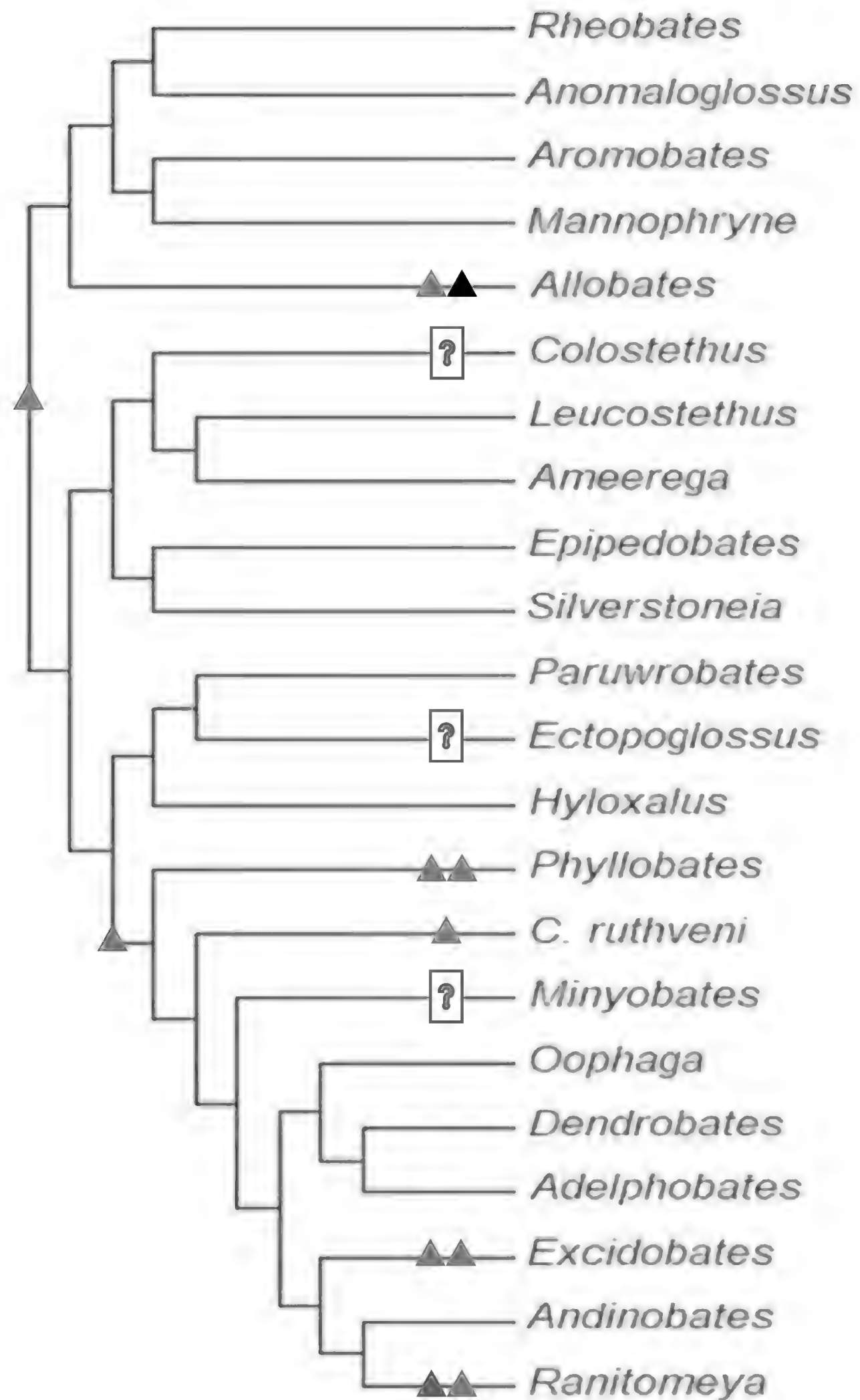
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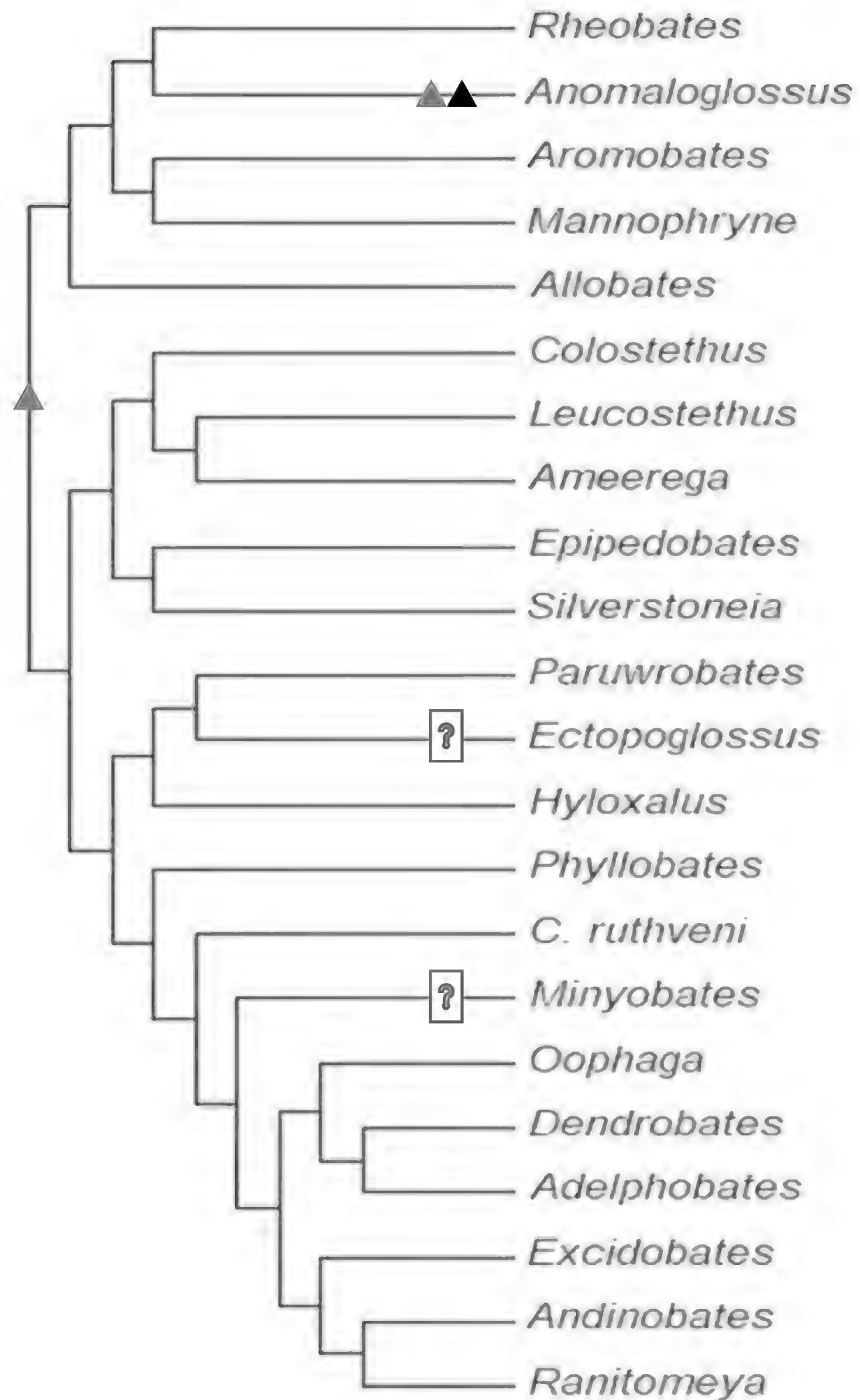
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93. Larval caudal coloration

- ▲ 0. vertically striped
- ▲ 1. scattered melanophores clumped to form diffuse blotches
- ▲ 2. Evenly pigmented

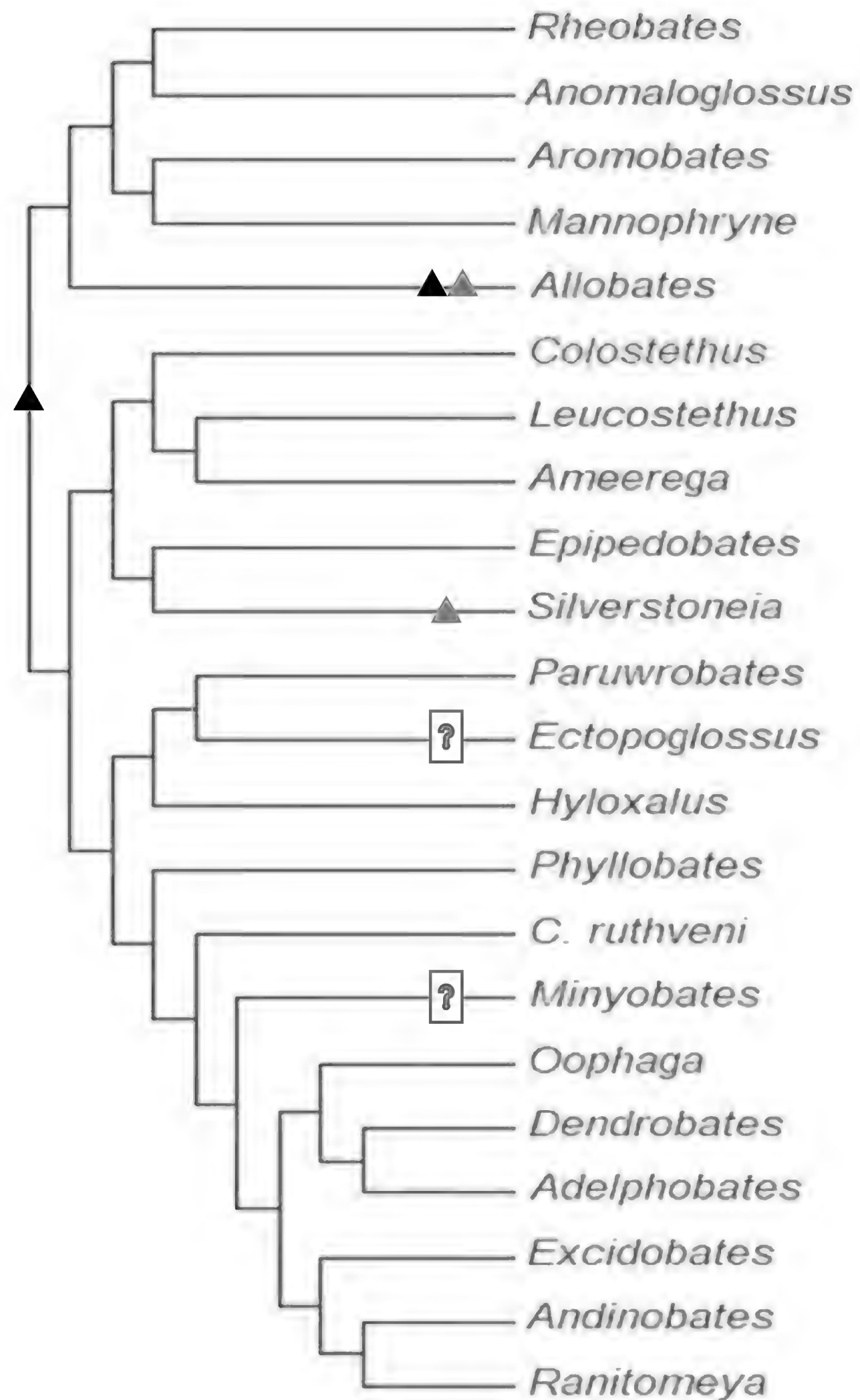
Nonadditive.



94. Larval oral disc occurrence

▲ 0. absent

▲ 1. present



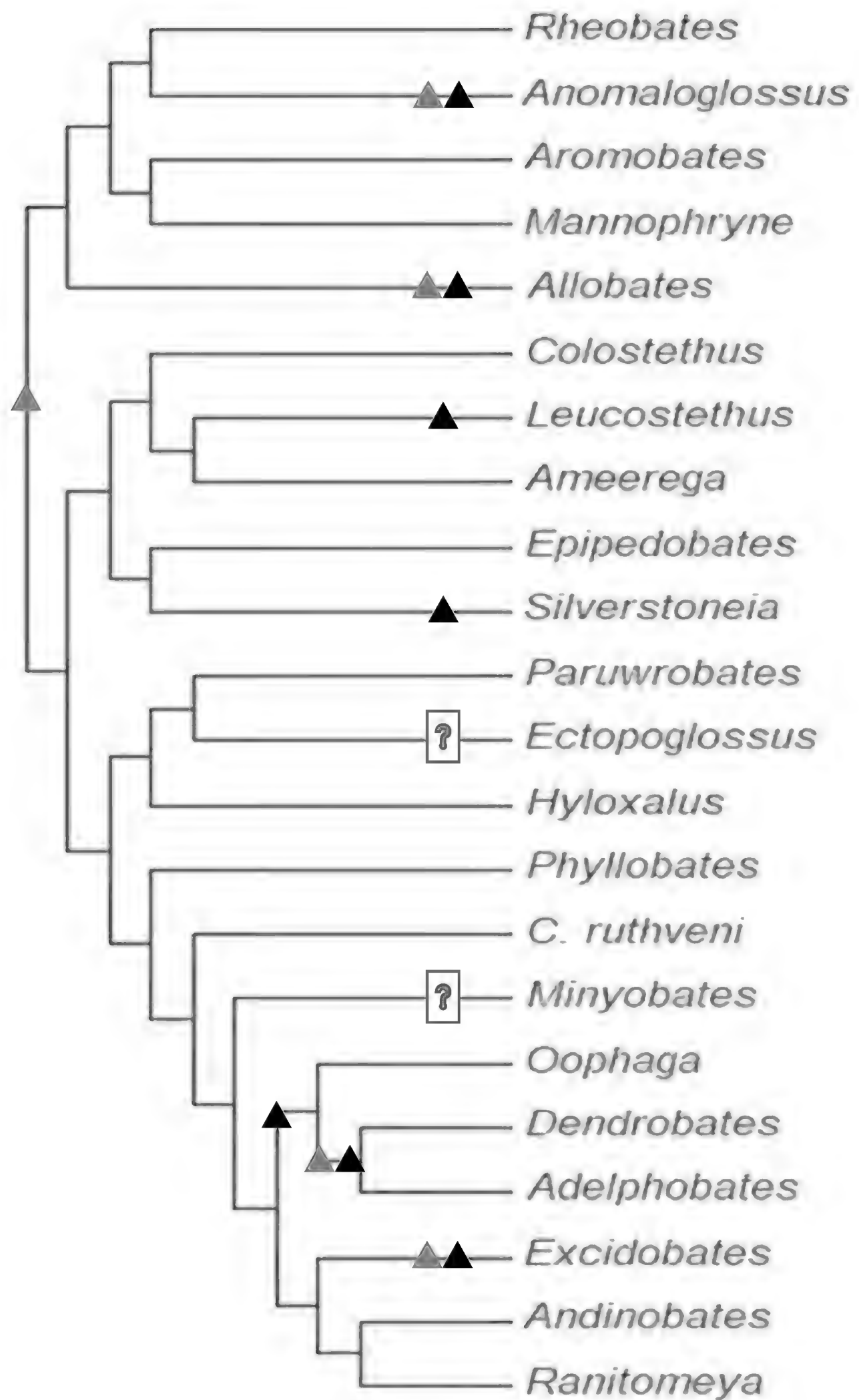
95. Larval oral disc morphology

▲ 0. "normal"

▲ 1. umbelliform

▲ 2. Suctorial

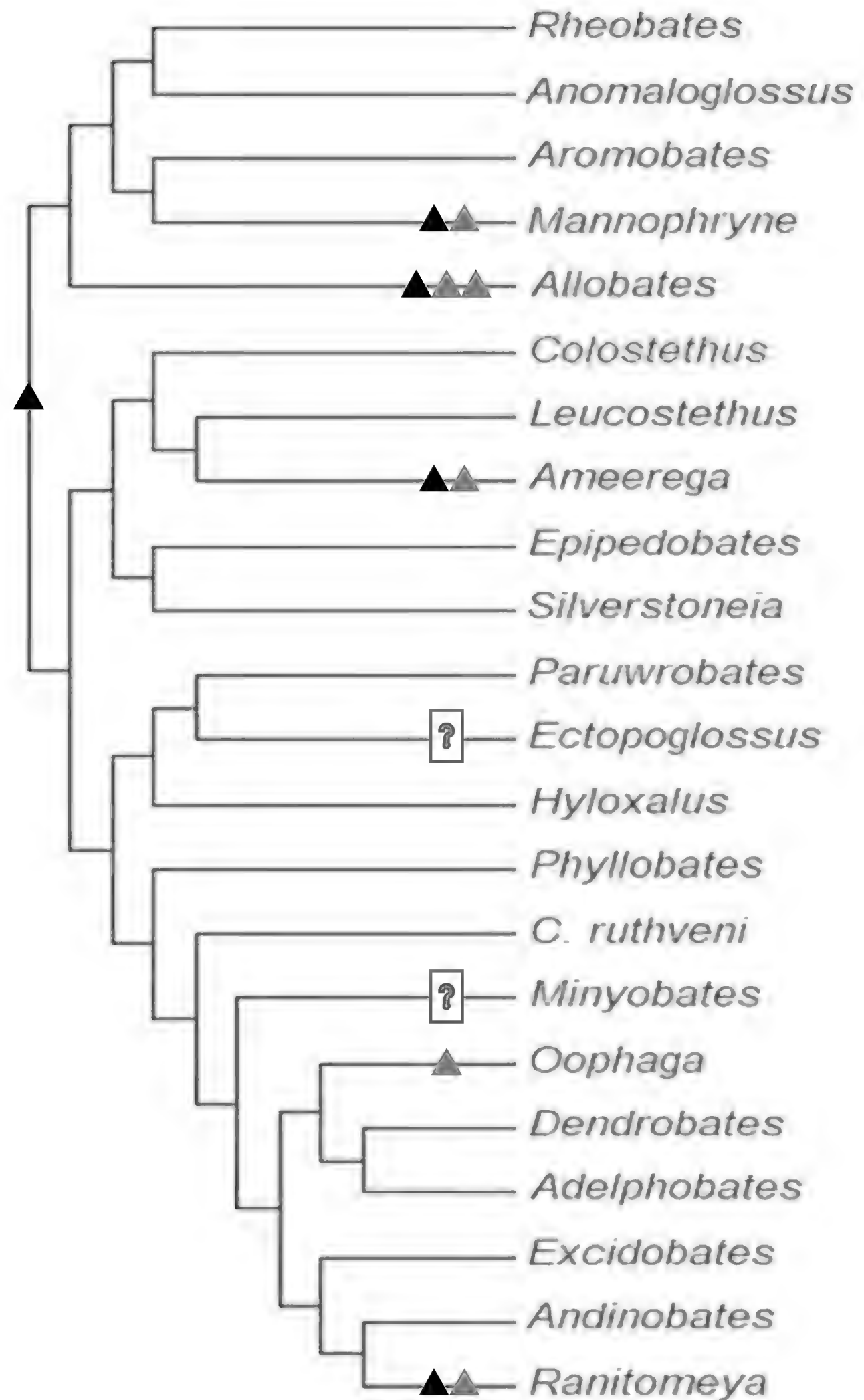
Nonadditive.



96. Lateral indentation of larval oral disc

▲ 0. absent (not emarginated)

▲ 1. present (emarginated)



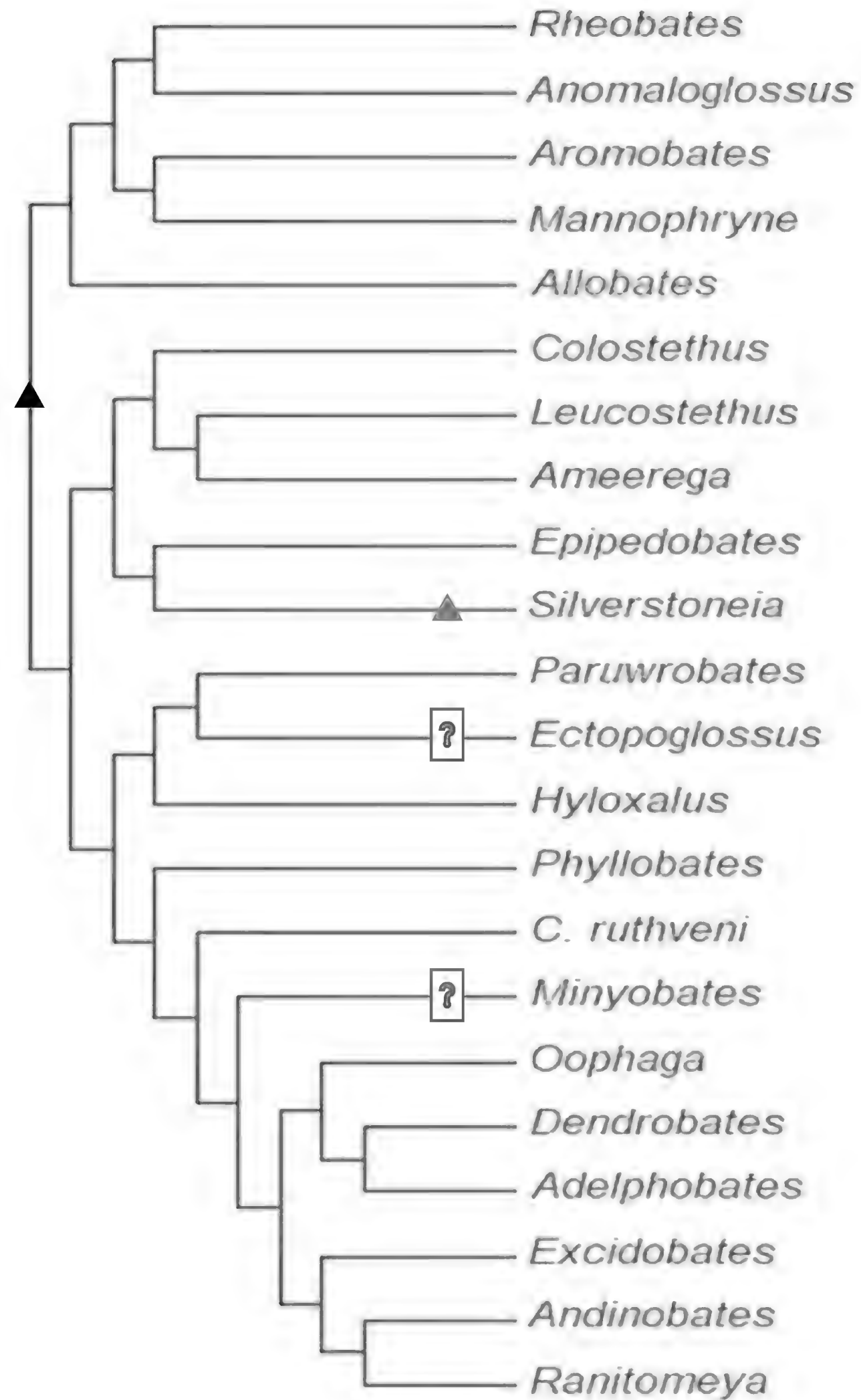
97. Larval marginal labial papillae size

▲ 0. short

▲ 1. enlarged

▲ 2. greatly enlarged

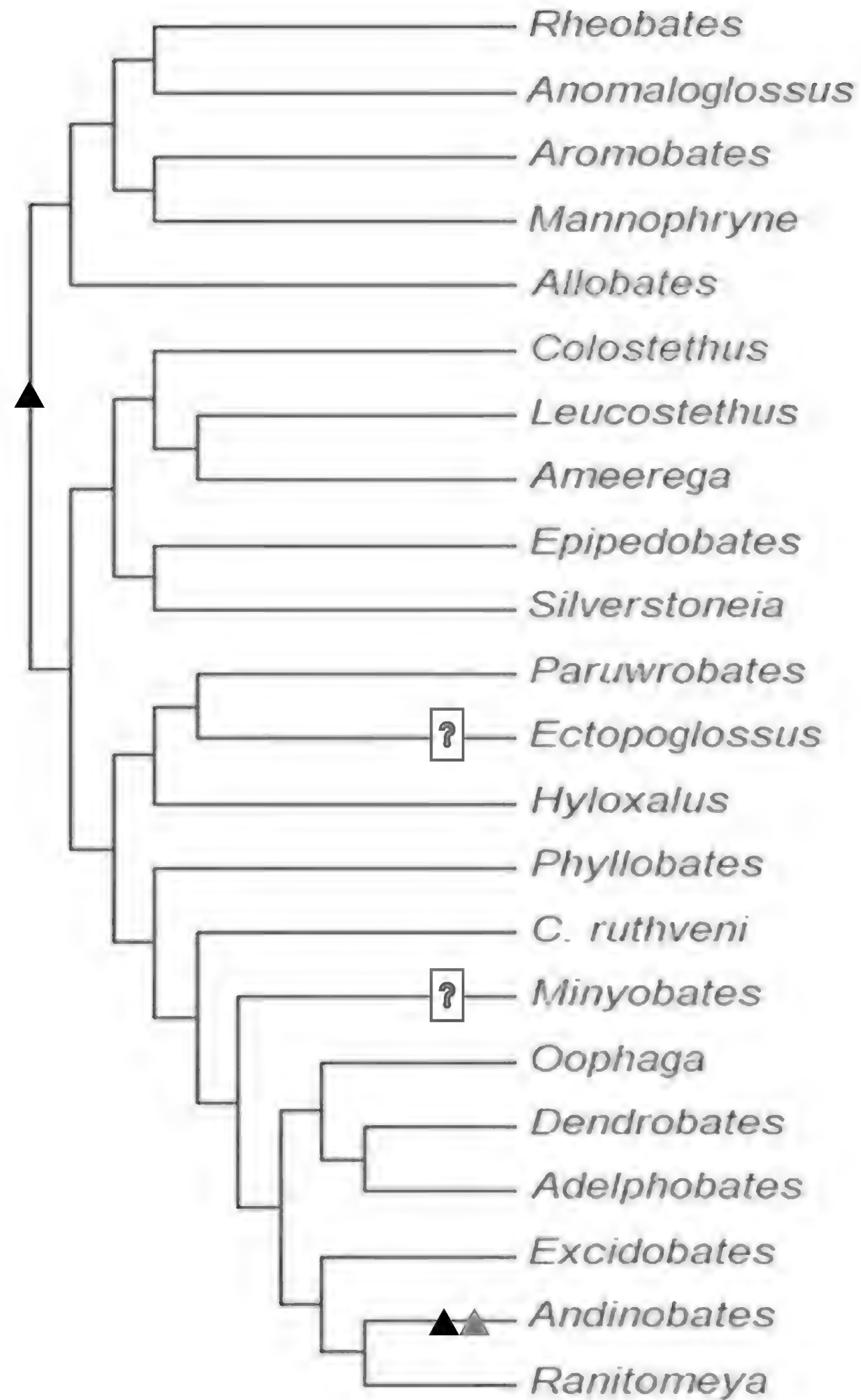
Additive.



98. Submarginal papillae of larval oral disc

▲ 0. absent

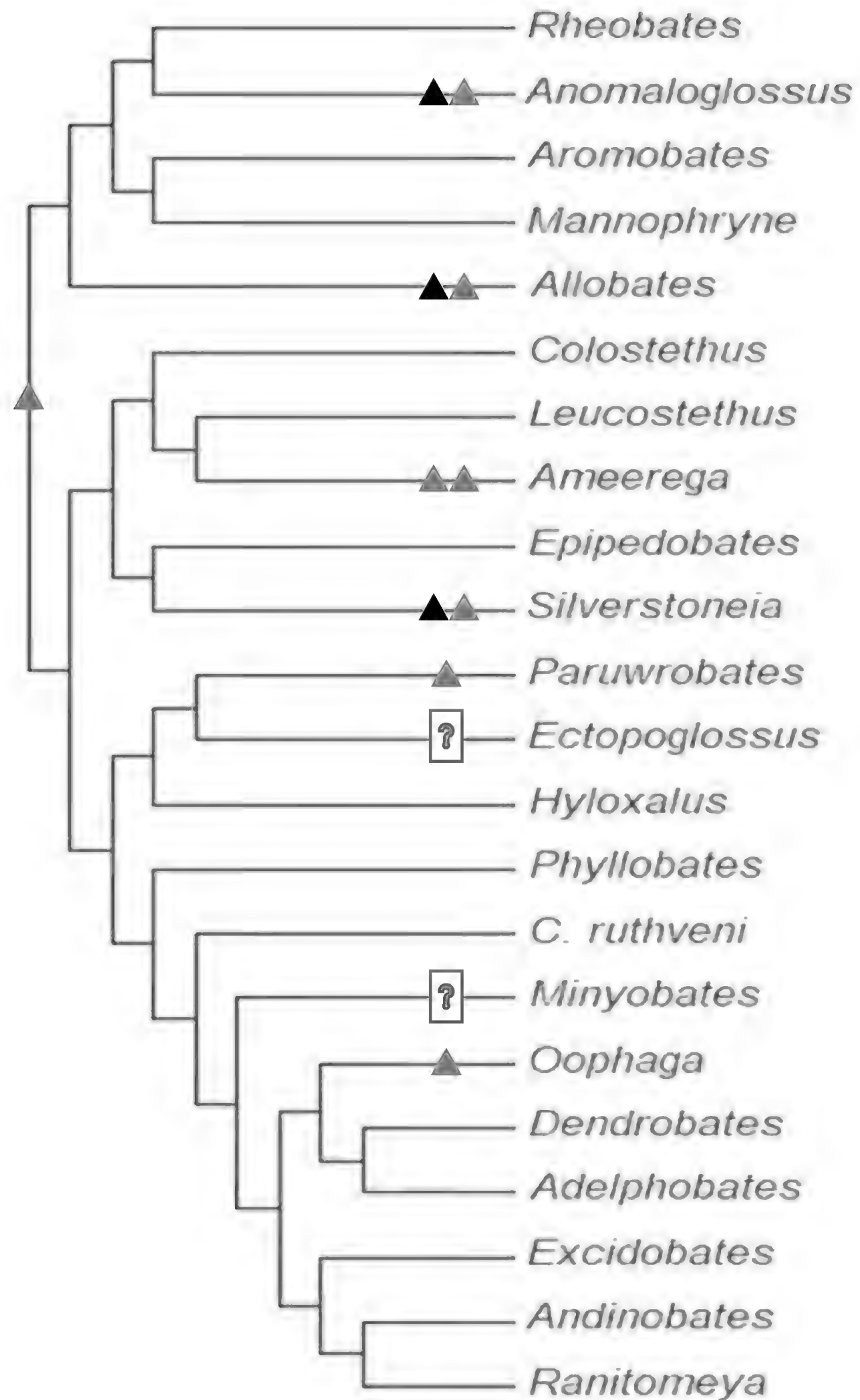
▲ 1. present



99. Median gap in larval marginal papillae of lower labium (PL-gap)

▲ 0. absent

▲ 1. present



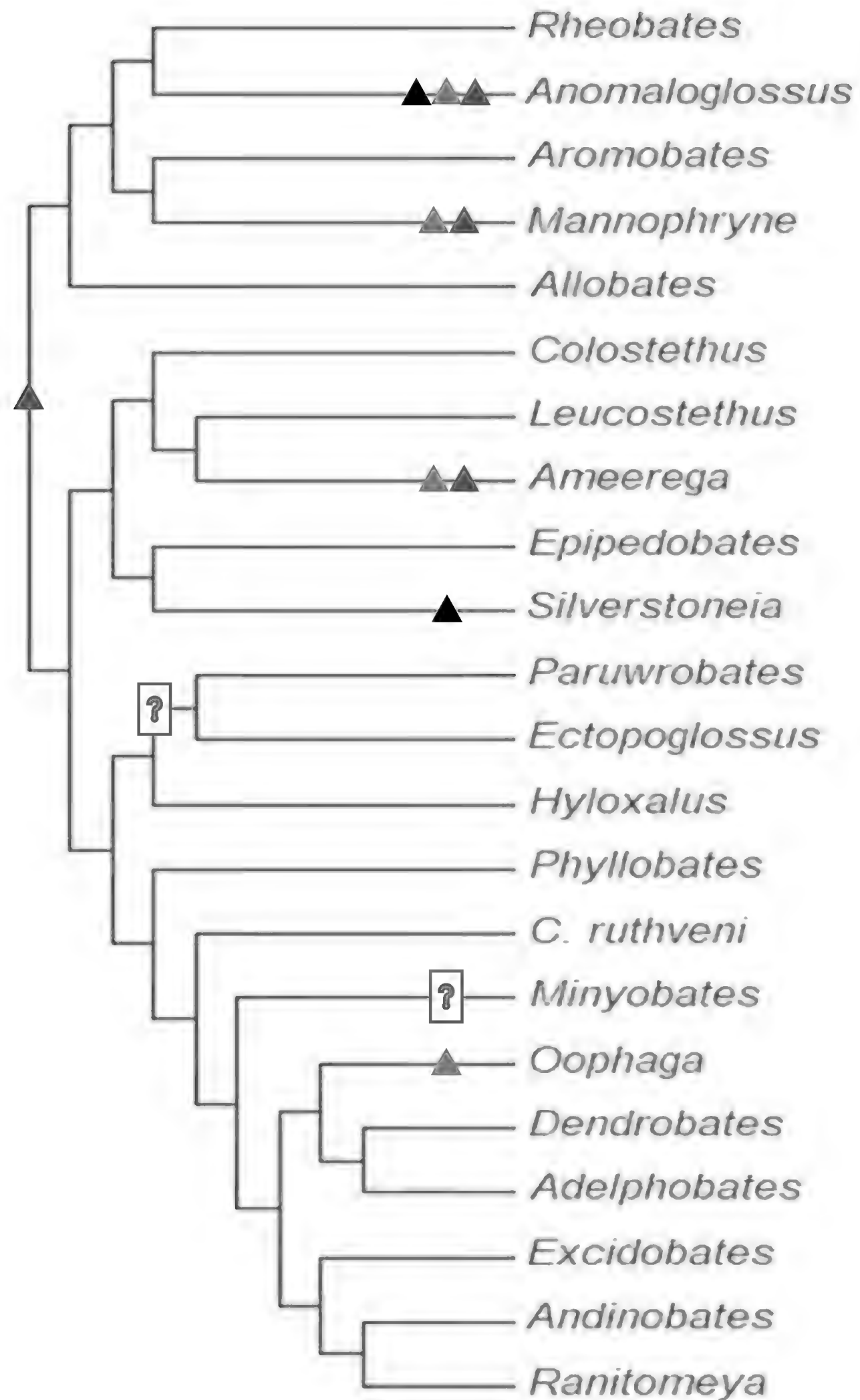
100. Anterior larval keratodont rows

▲ 0. zero

▲ 1. one

▲ 2. two

Additive.



101. Posterior larval keratodont rows

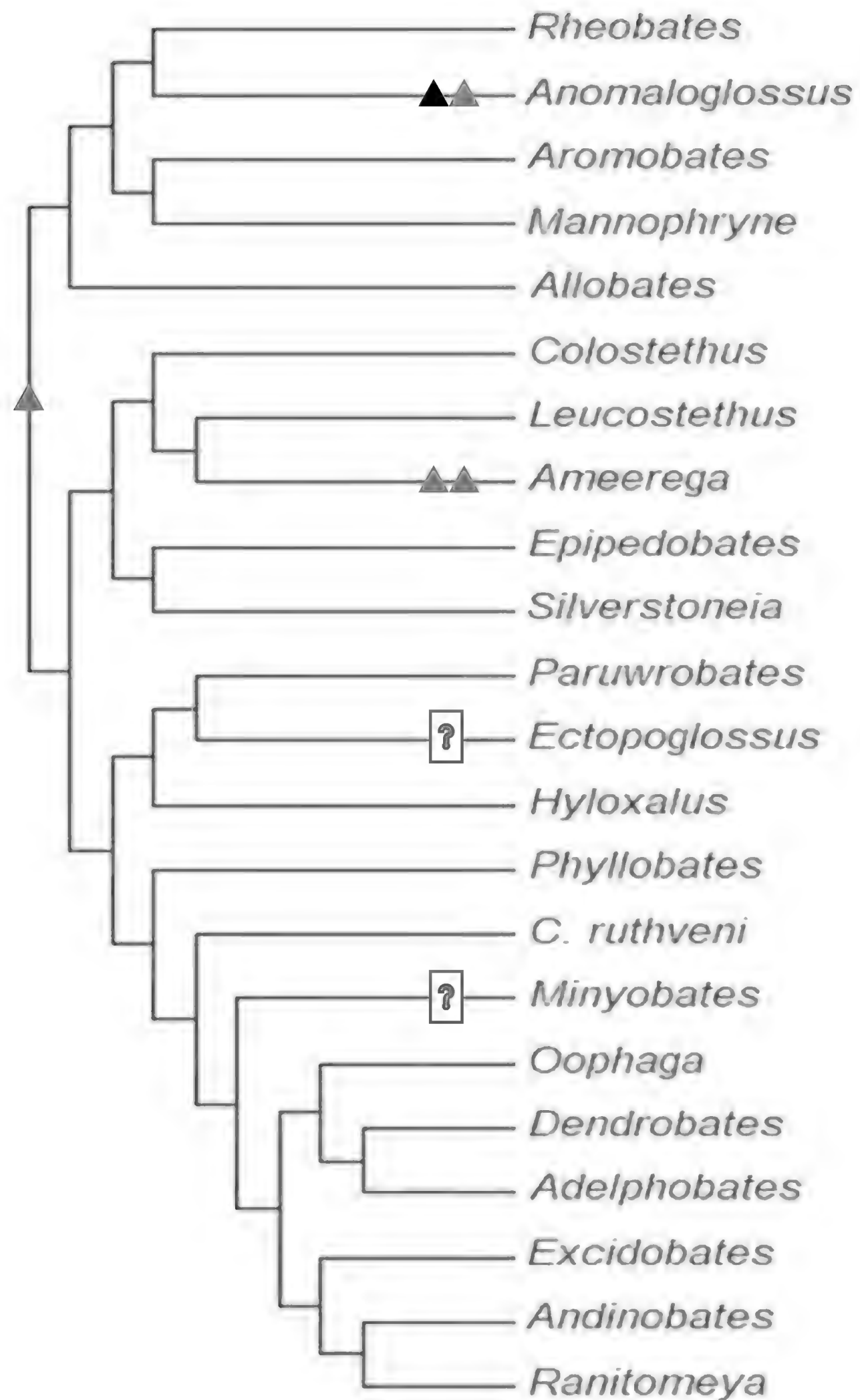
▲ 0. zero

▲ 1. one

▲ 2. two

▲ 3. Three

Additive



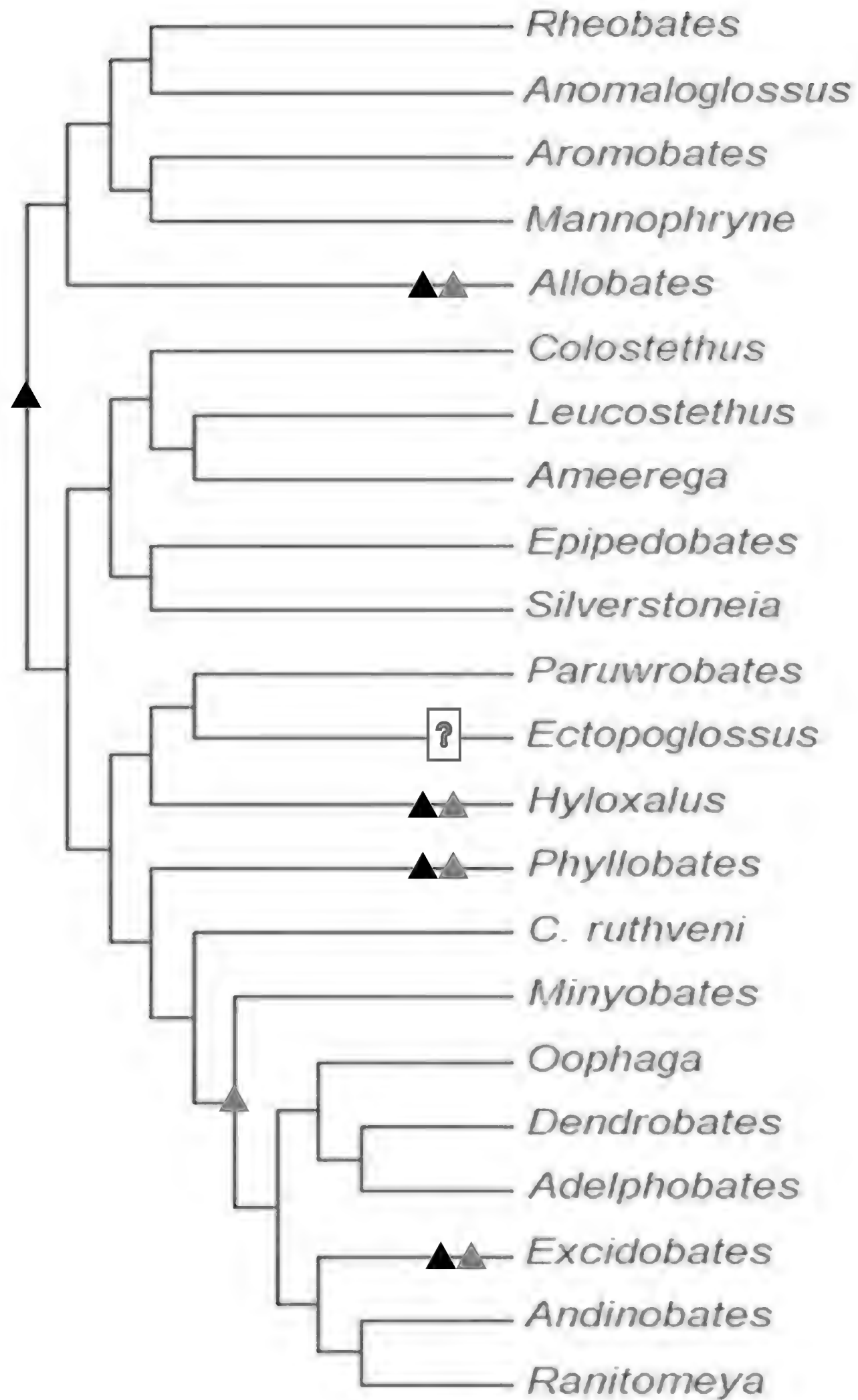
102. Larval jaw sheaths

▲ 0. absent

▲ 1. lower only, not keratinized

▲ 2. entire, keratinized

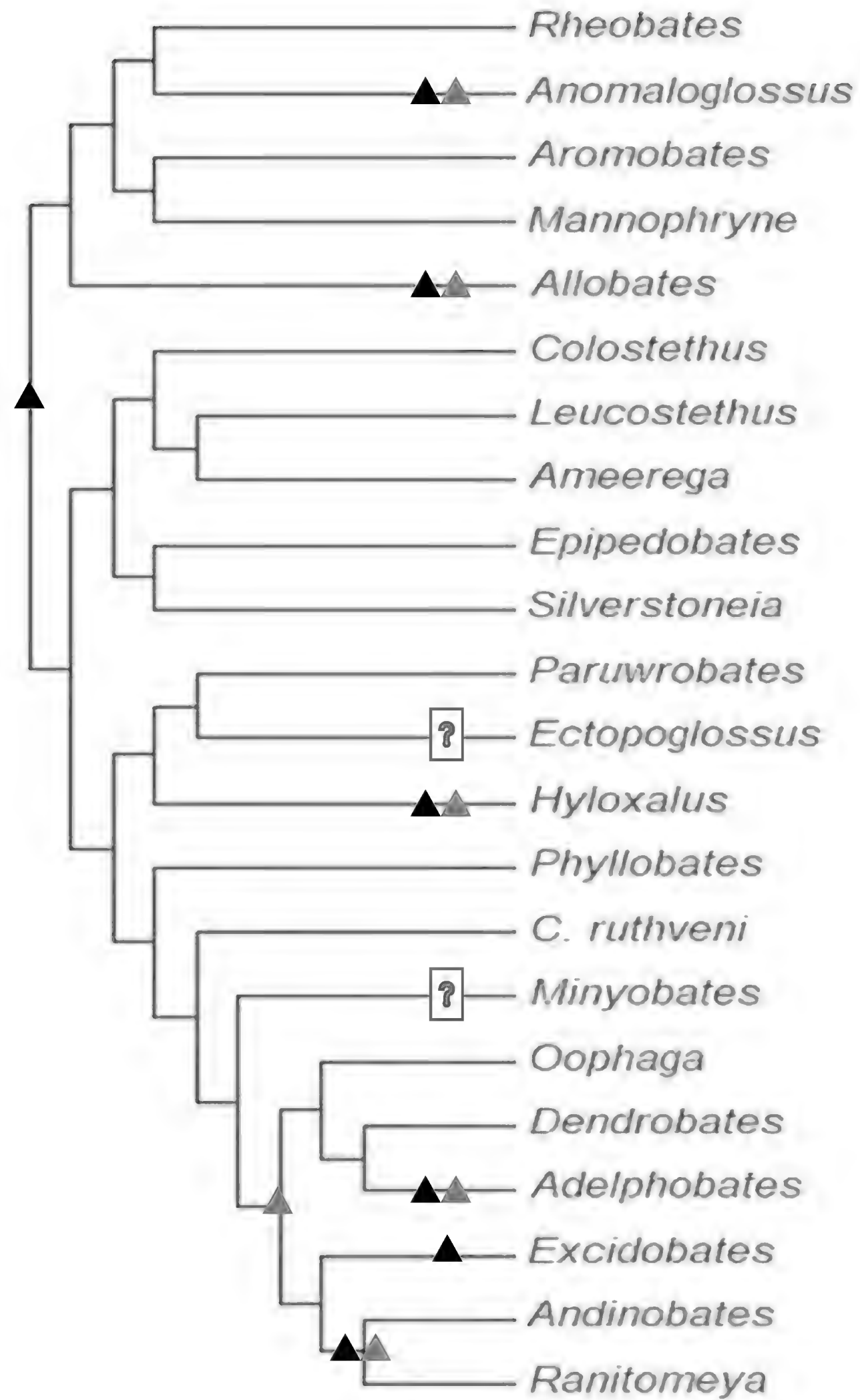
Additive.



103. Larval upper jaw sheath shape

▲ 0. W-shaped

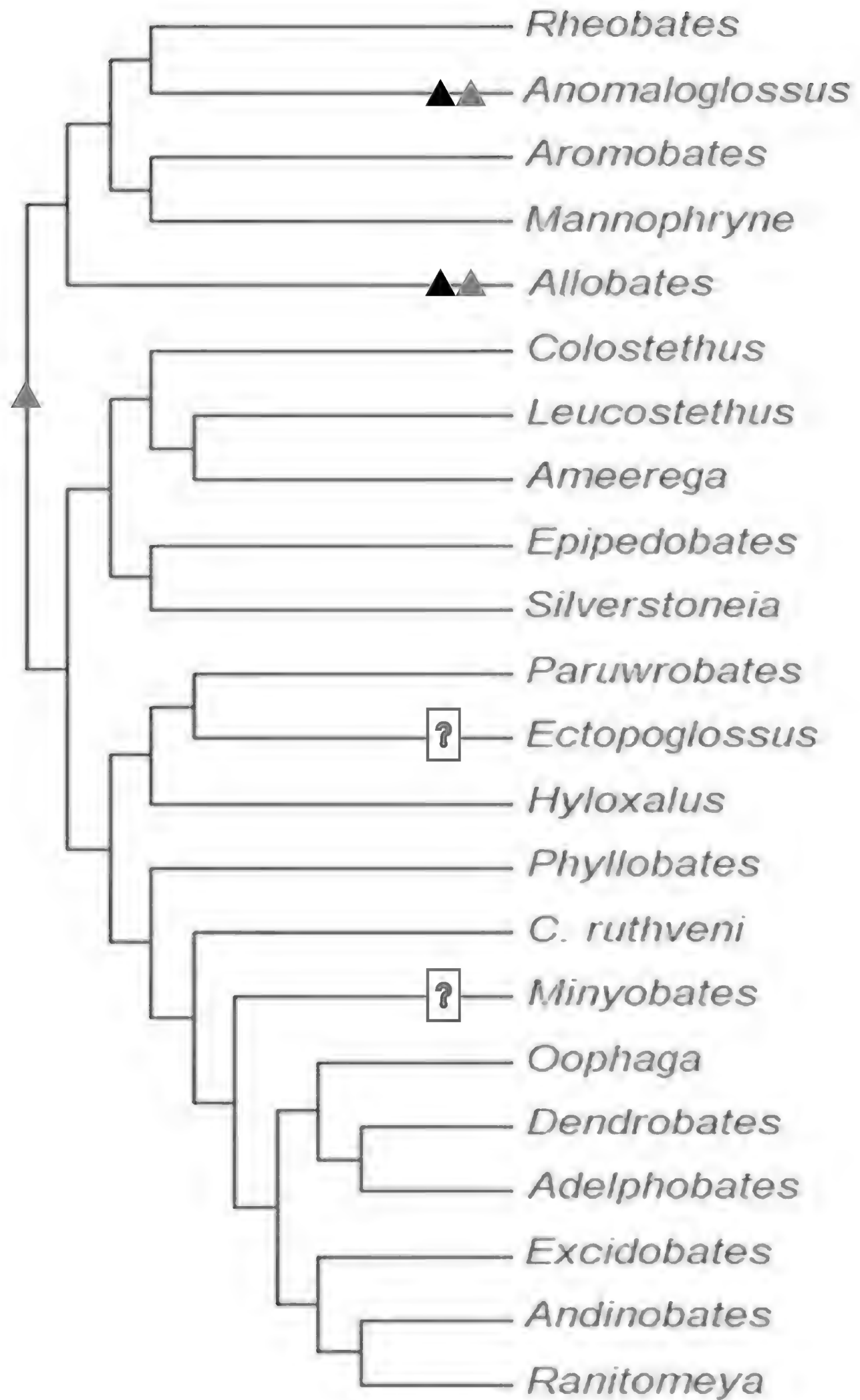
▲ 1. U-shaped



104. Larval vent tube position

▲ 0. dextral

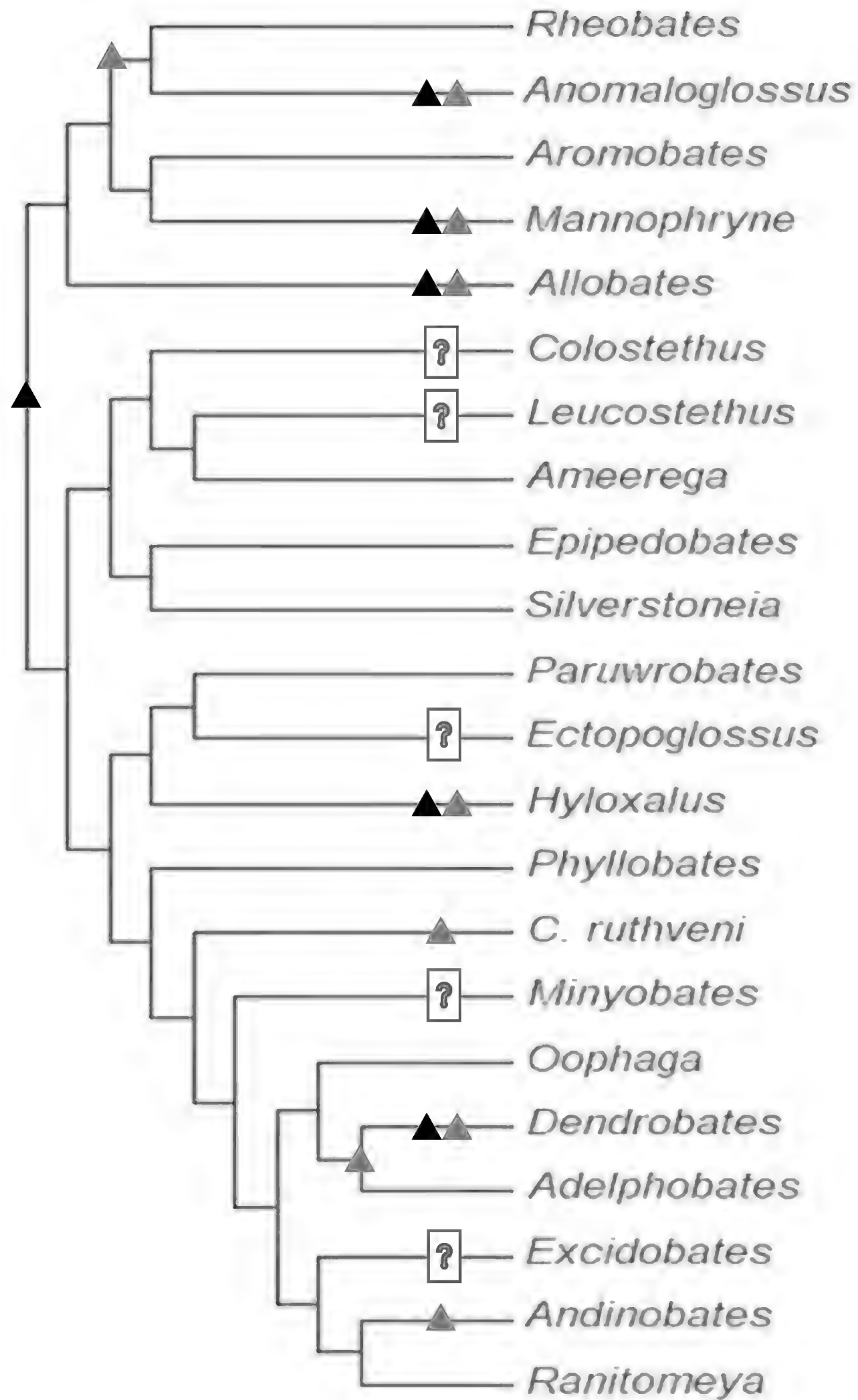
▲ 1. median



105. Spiracle

▲ 0. absent

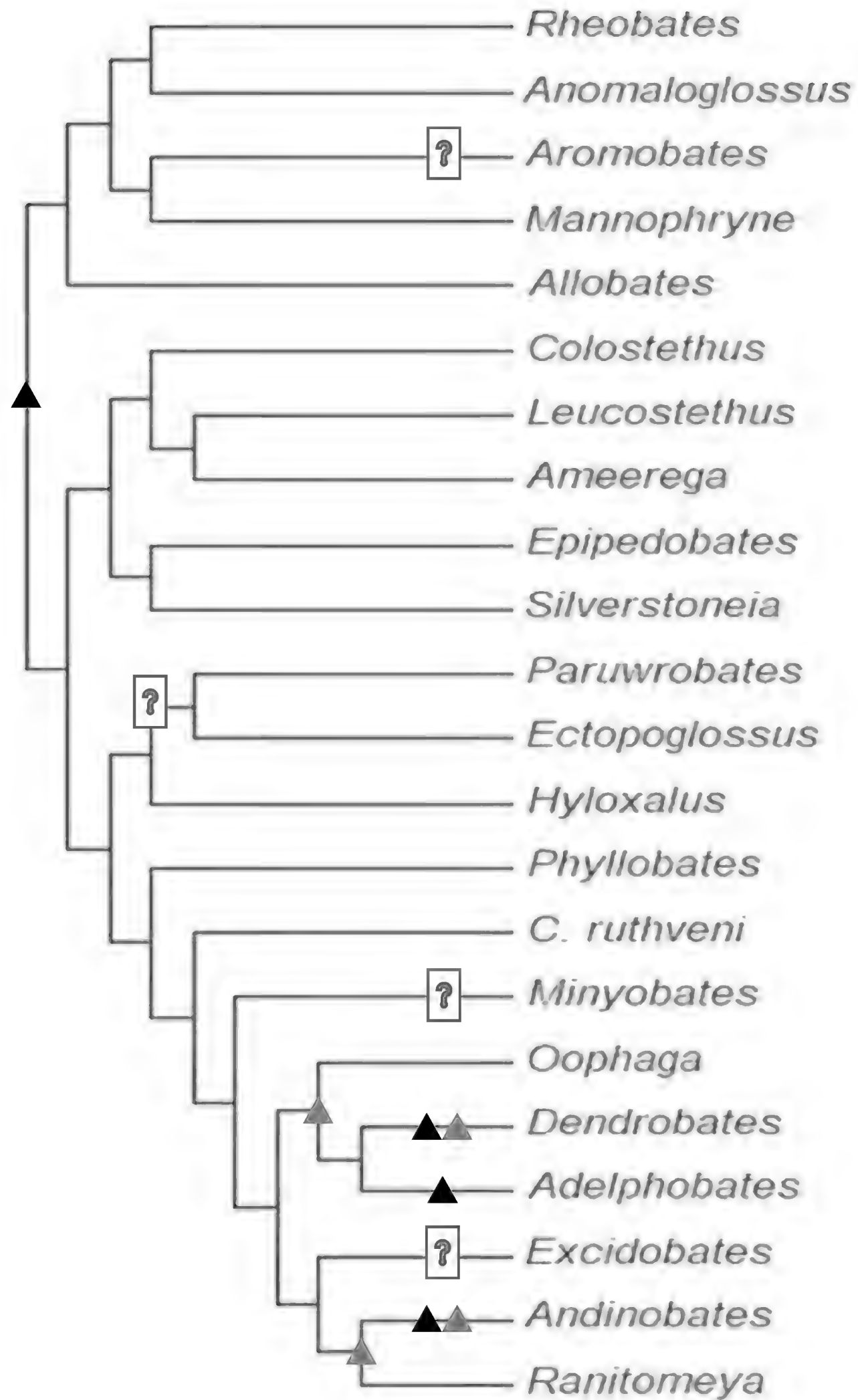
▲ 1. present



106. Lateral line stitches

▲ 0. absent

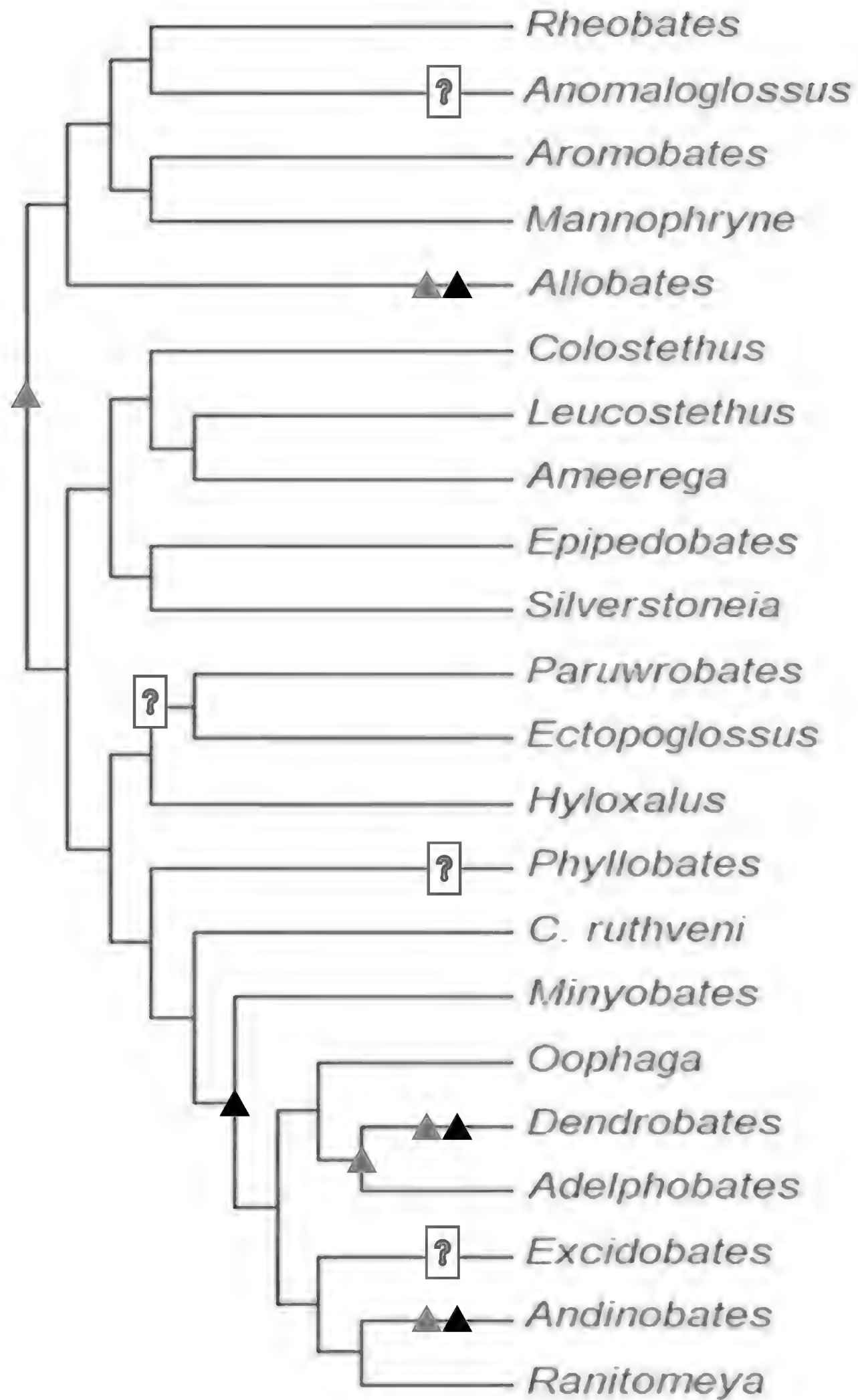
▲ 1. present



107. Larval gut morphology

▲ 0. long gut concealing other organs

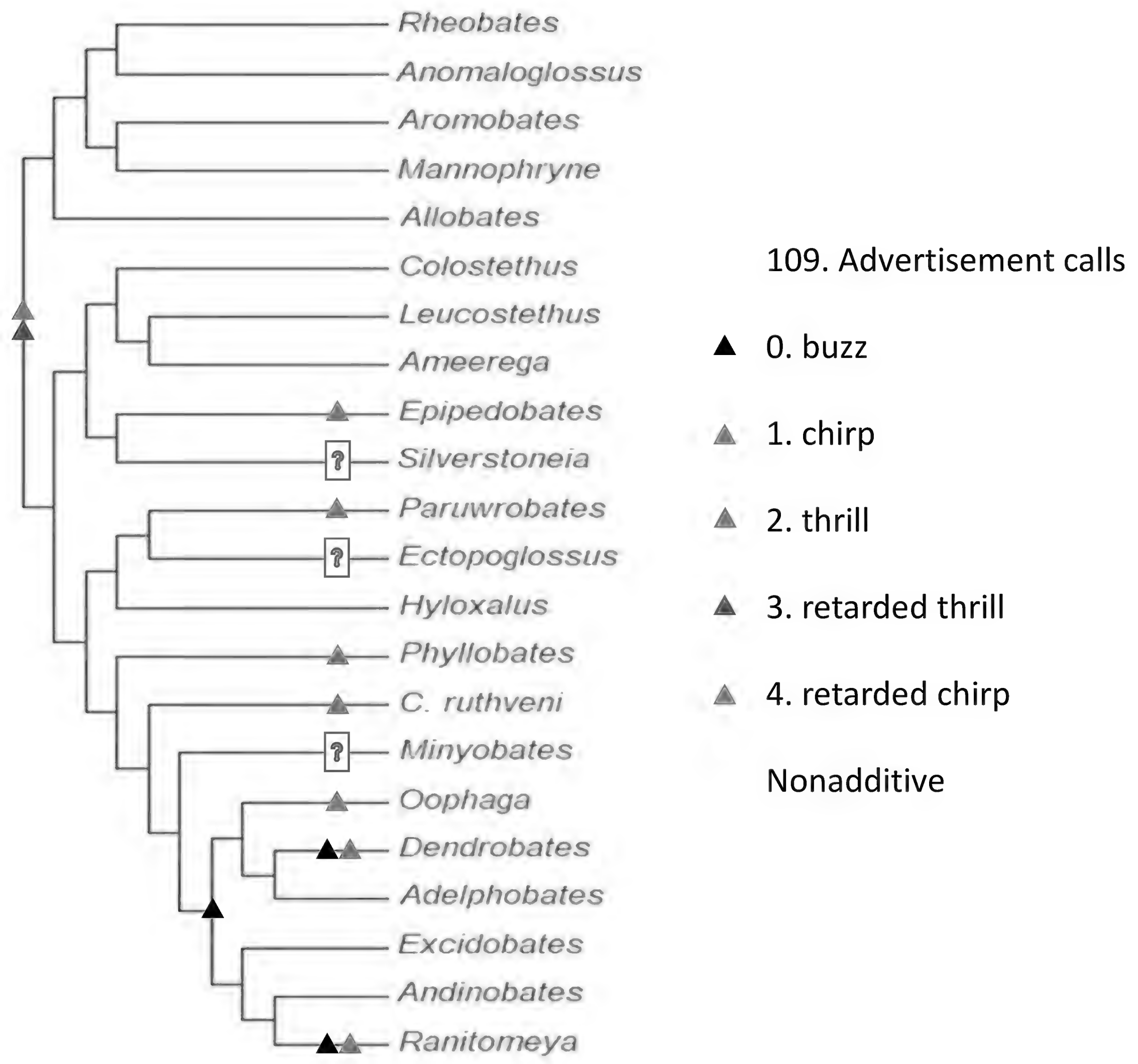
▲ 1. short gut revealing other organs

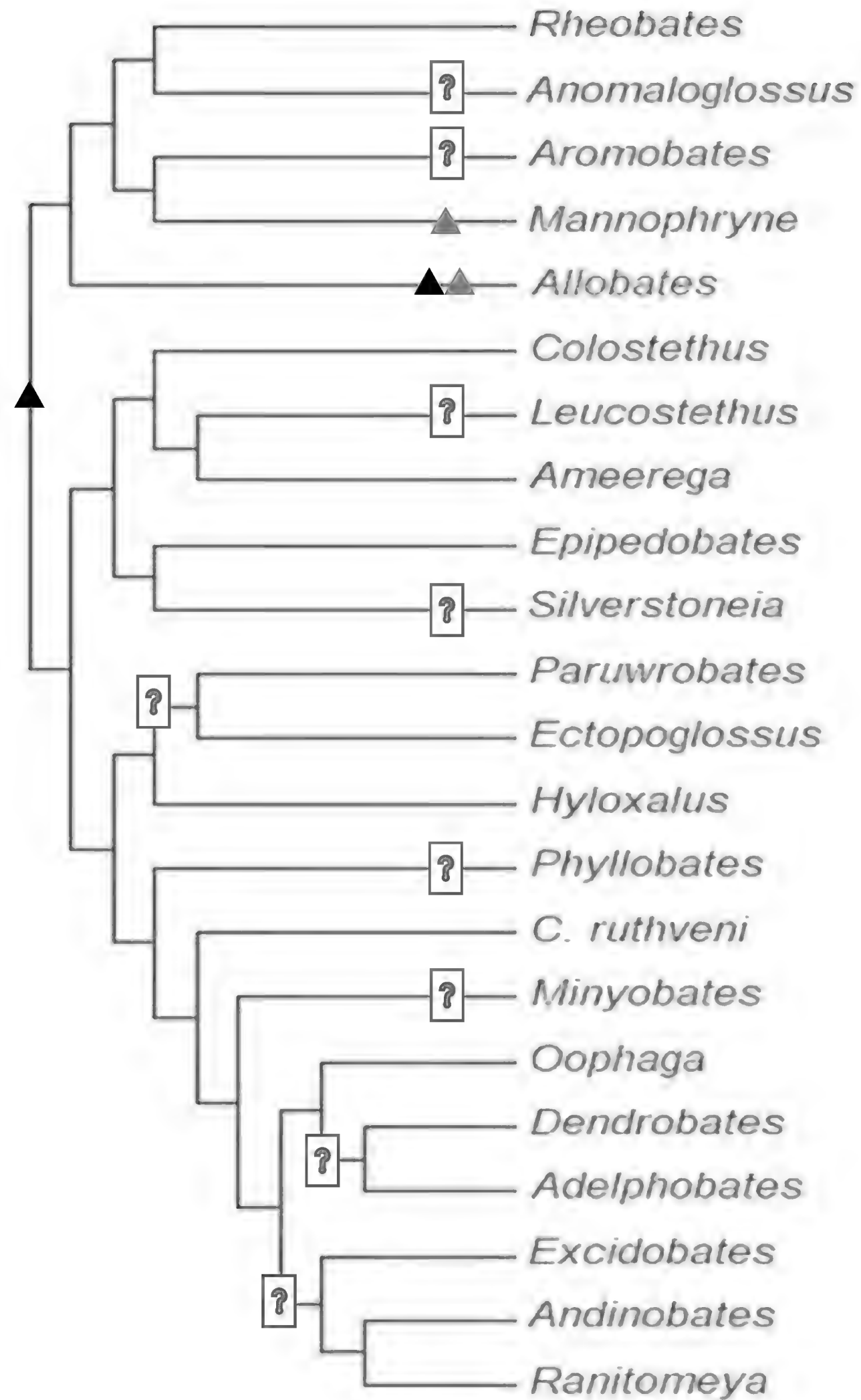


108. Projection on sagittal edge of rim
of larval external nares

▲ 0. absent (non projected)

▲ 1. present (projected)

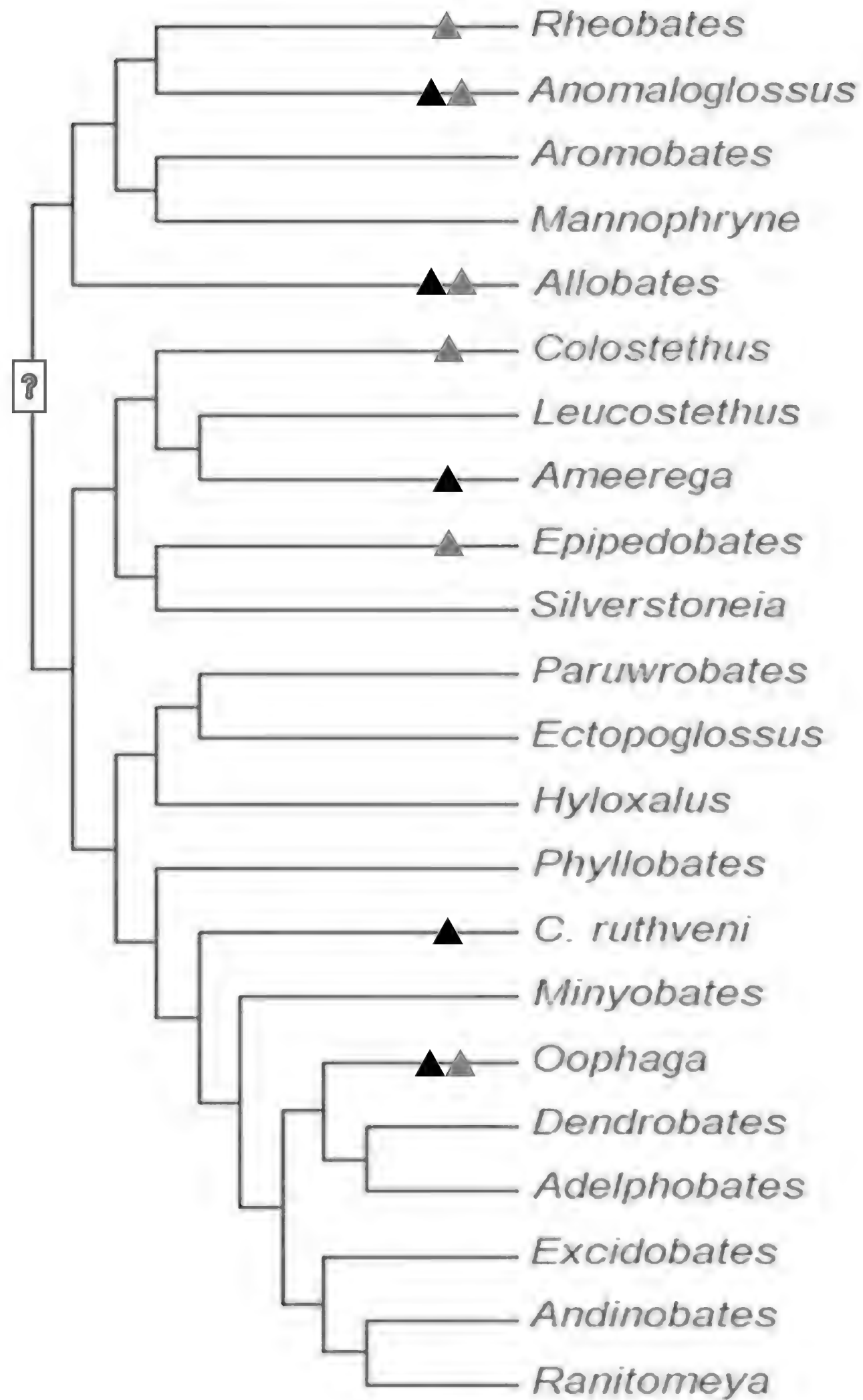




111. Male courtship: Jumping up and down

▲ 0. absent

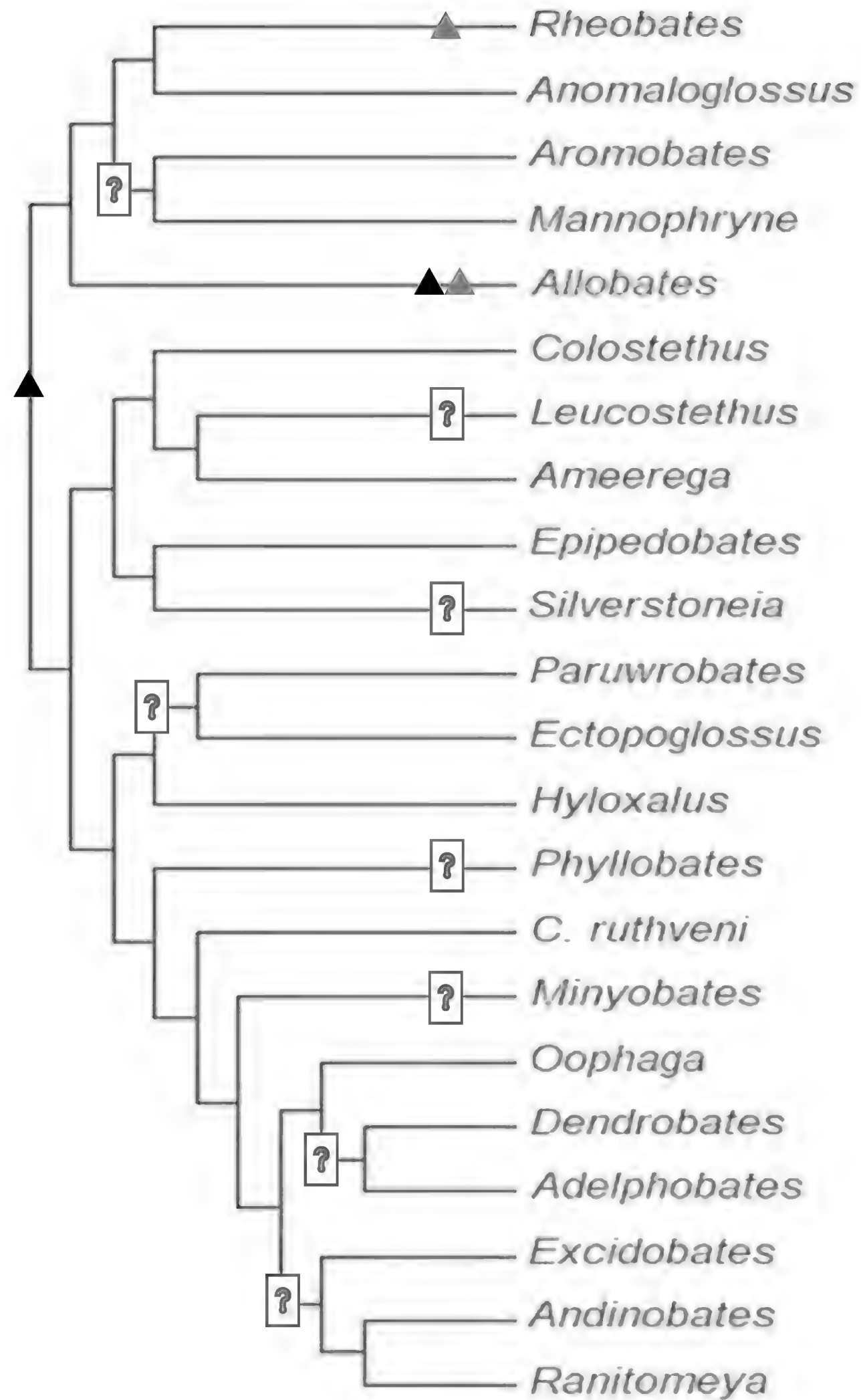
▲ 1. present



112. Female courtship: Crouching

▲ 0. absent

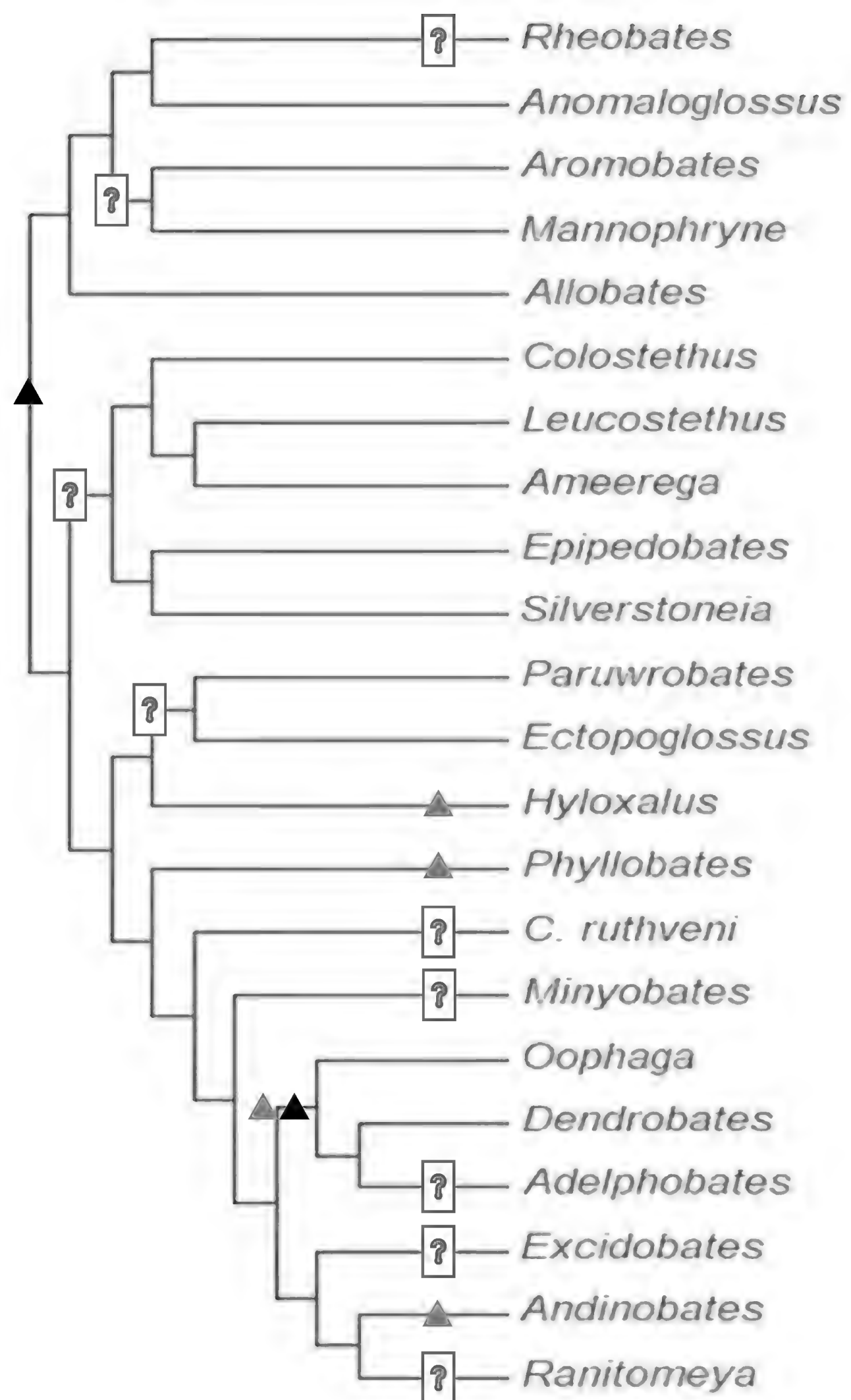
▲ 1. present



113. Female courtship: Sliding under male

▲ 0. absent

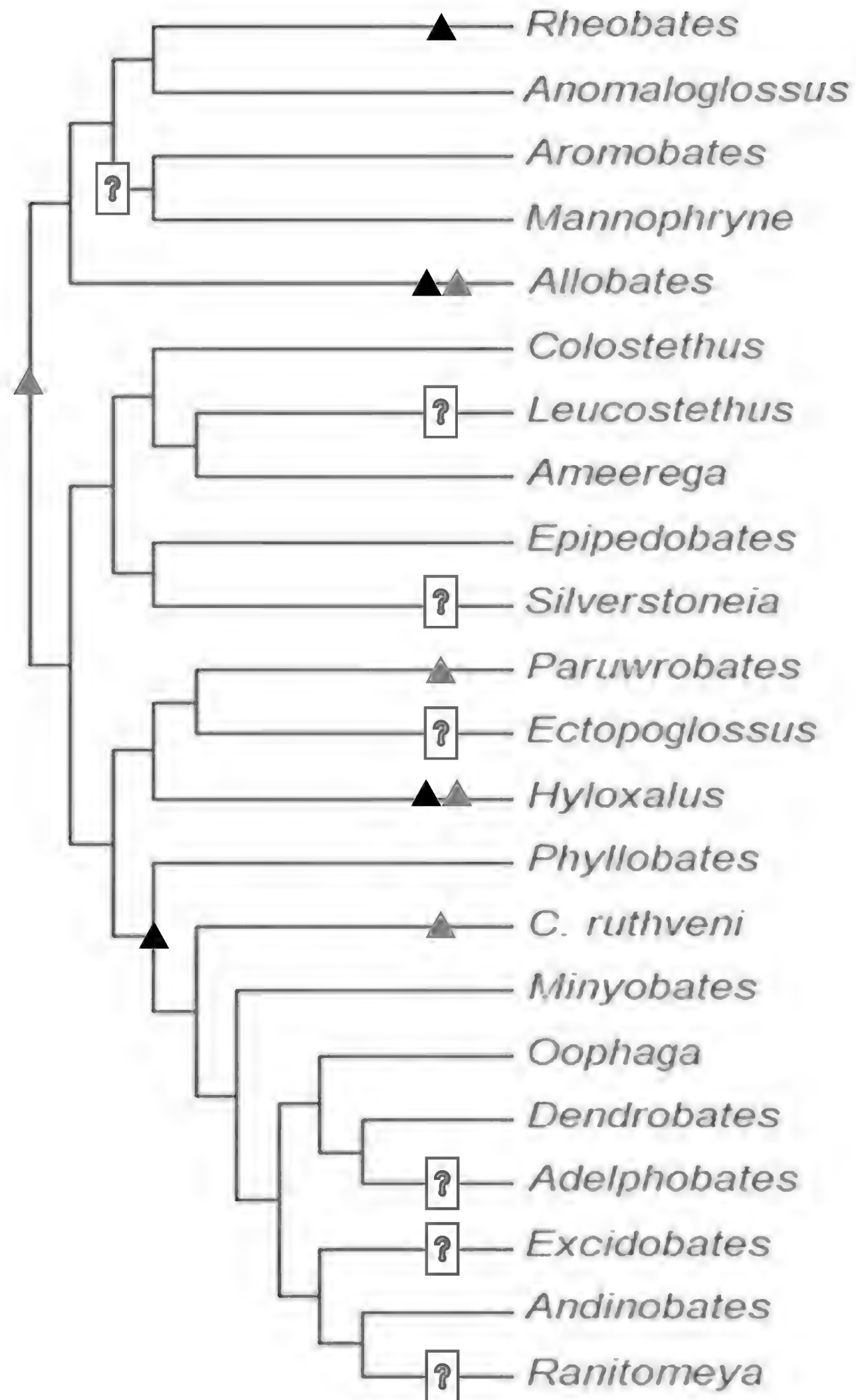
▲ 1. present



114. Timing of sperm deposition

▲ 0. after oviposition

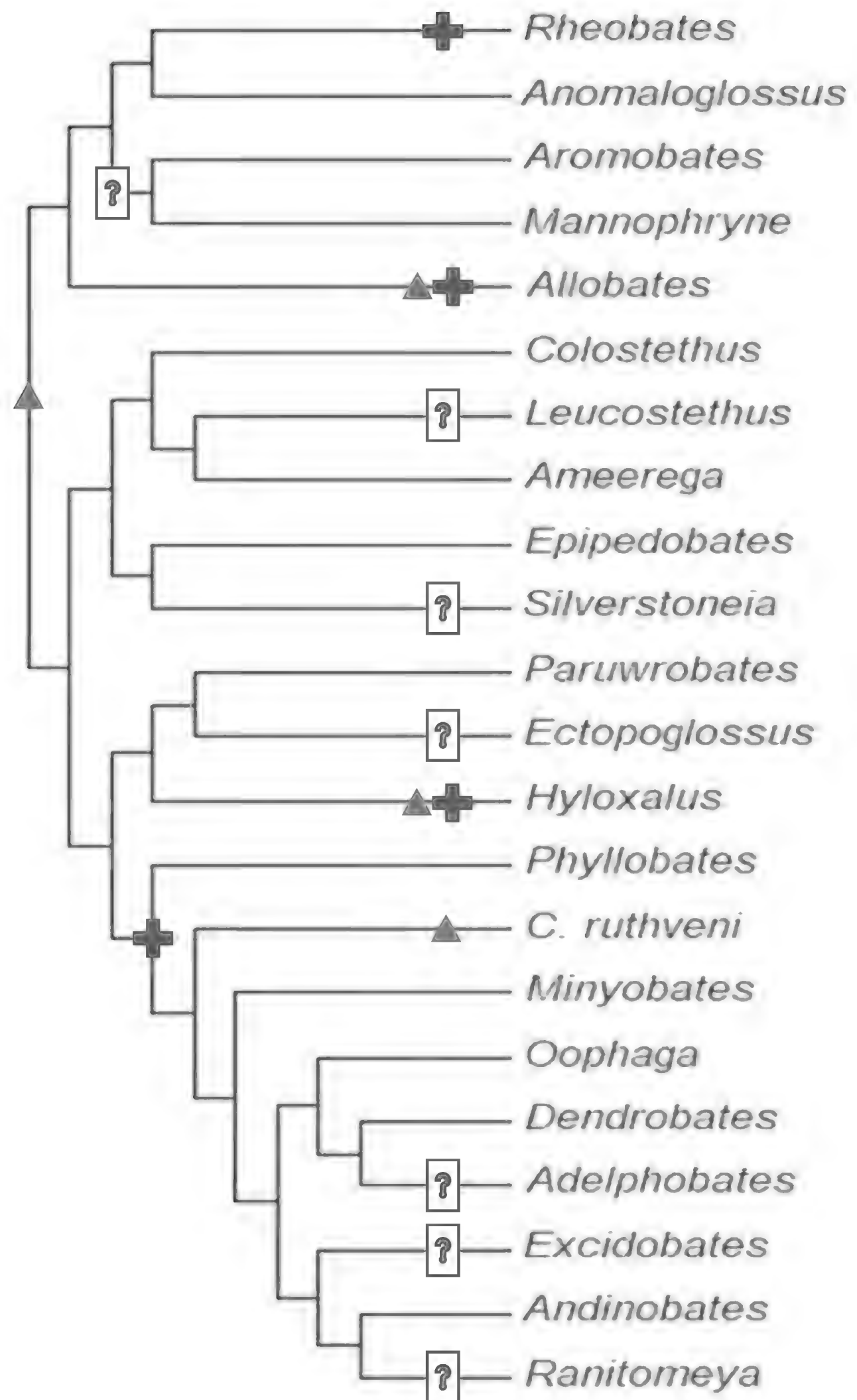
▲ 1. prior to oviposition



115. Reproductive amplexus
occurrence

▲ 0. absent

▲ 1. present

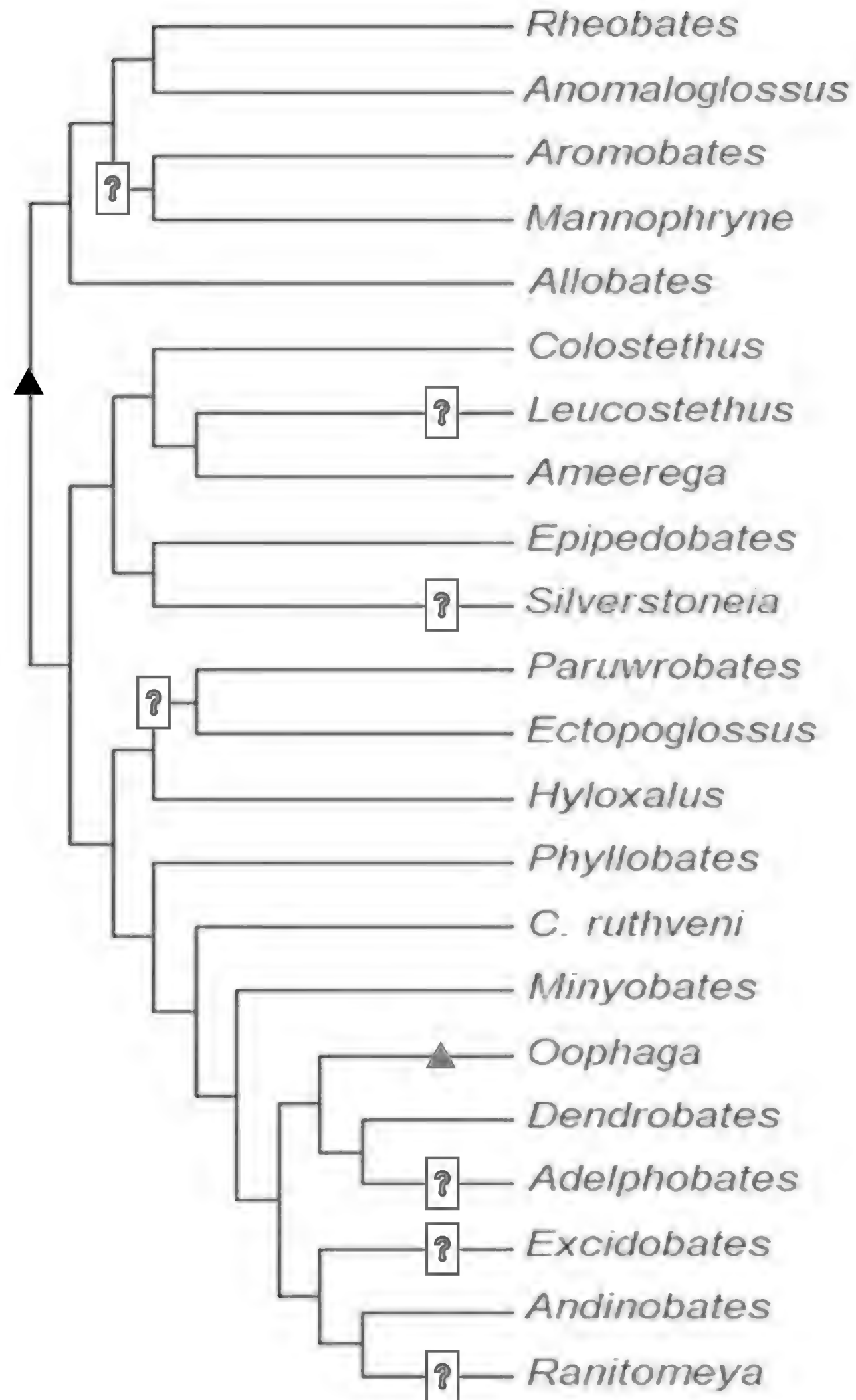


116. Reproductive amplexus position

▲ 0. axillary

▲ 1. cephalic

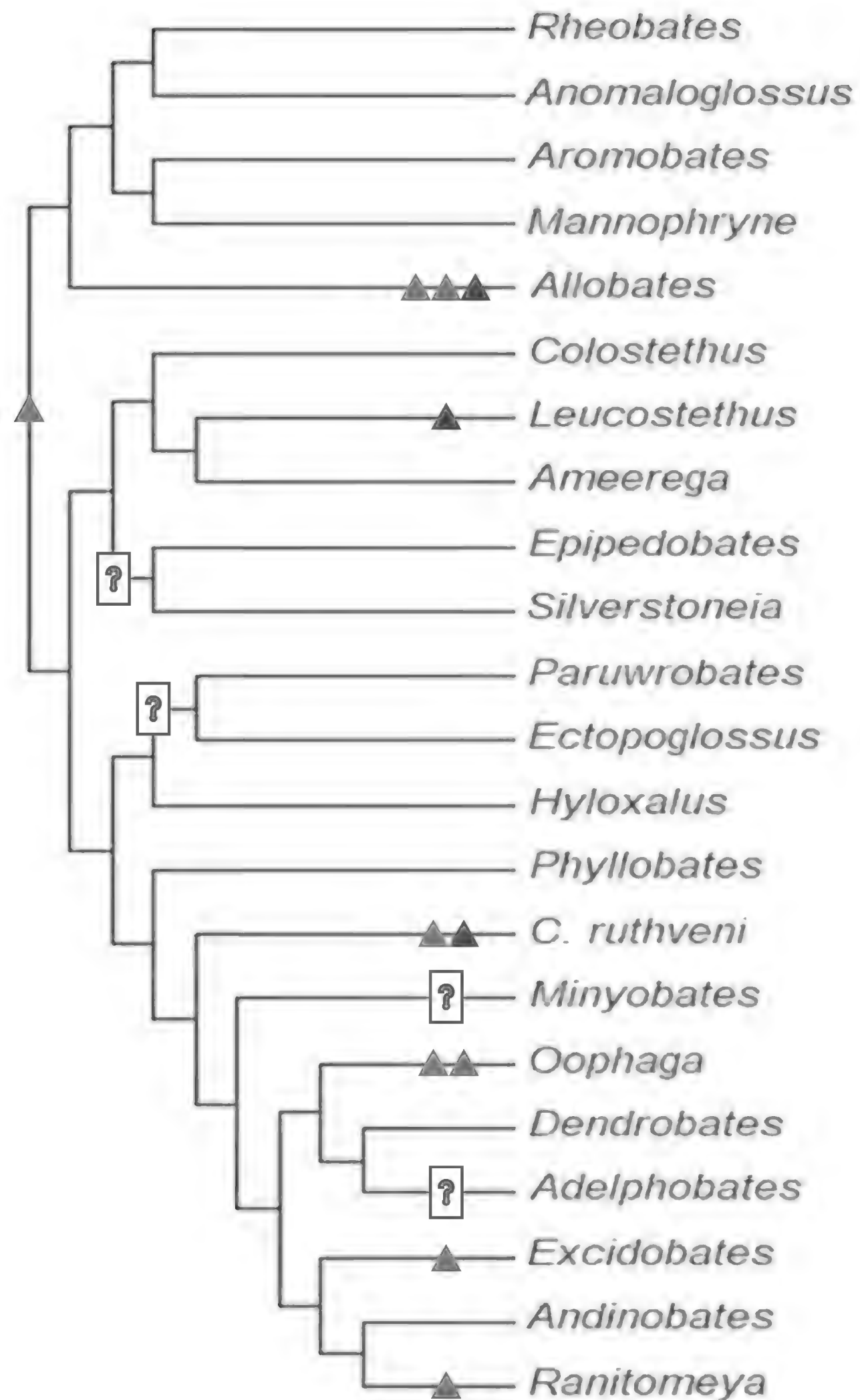
+ Not available



117. Cloaca-cloaca touching

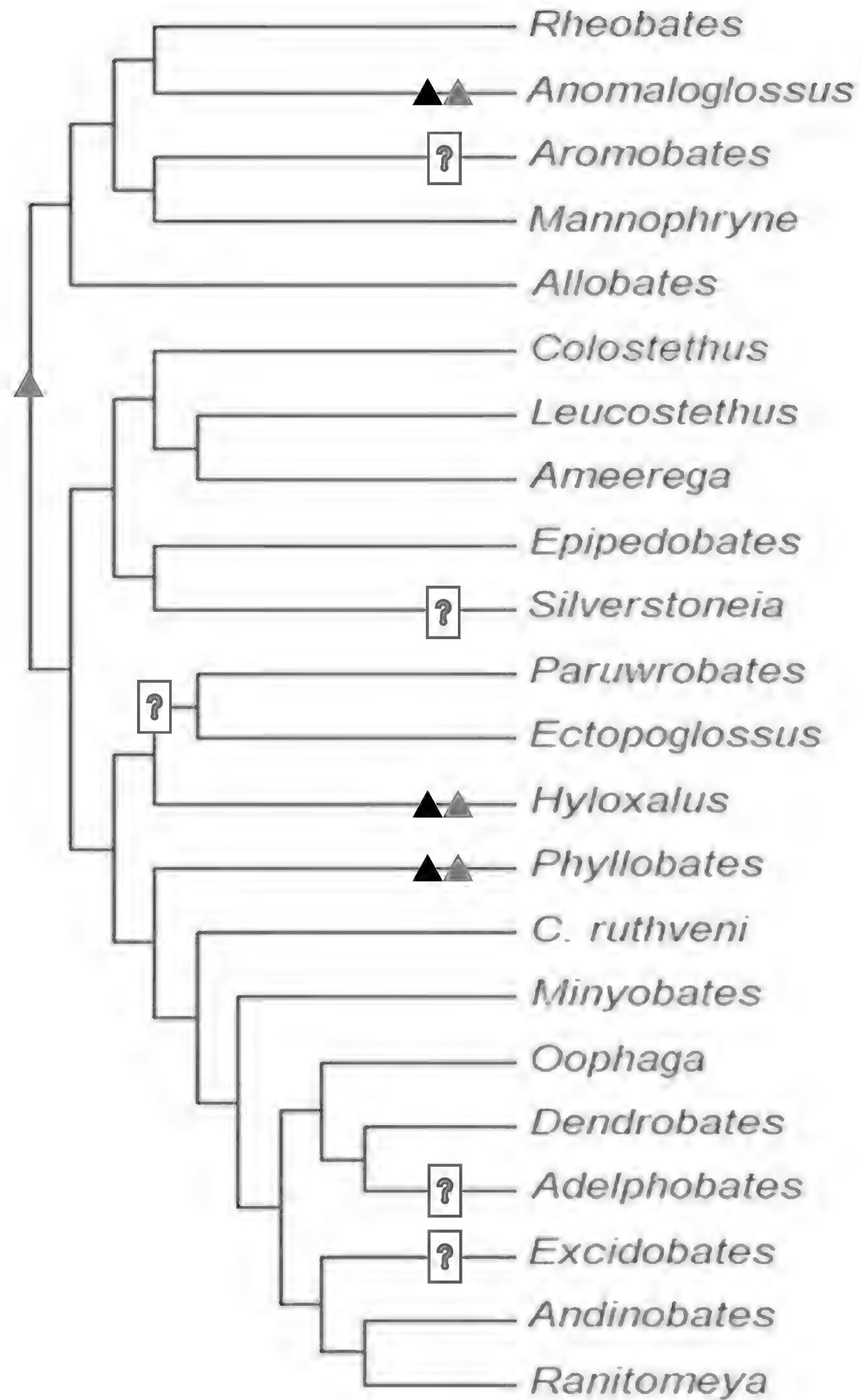
▲ 0. absent

▲ 1. present



118. Egg deposition site

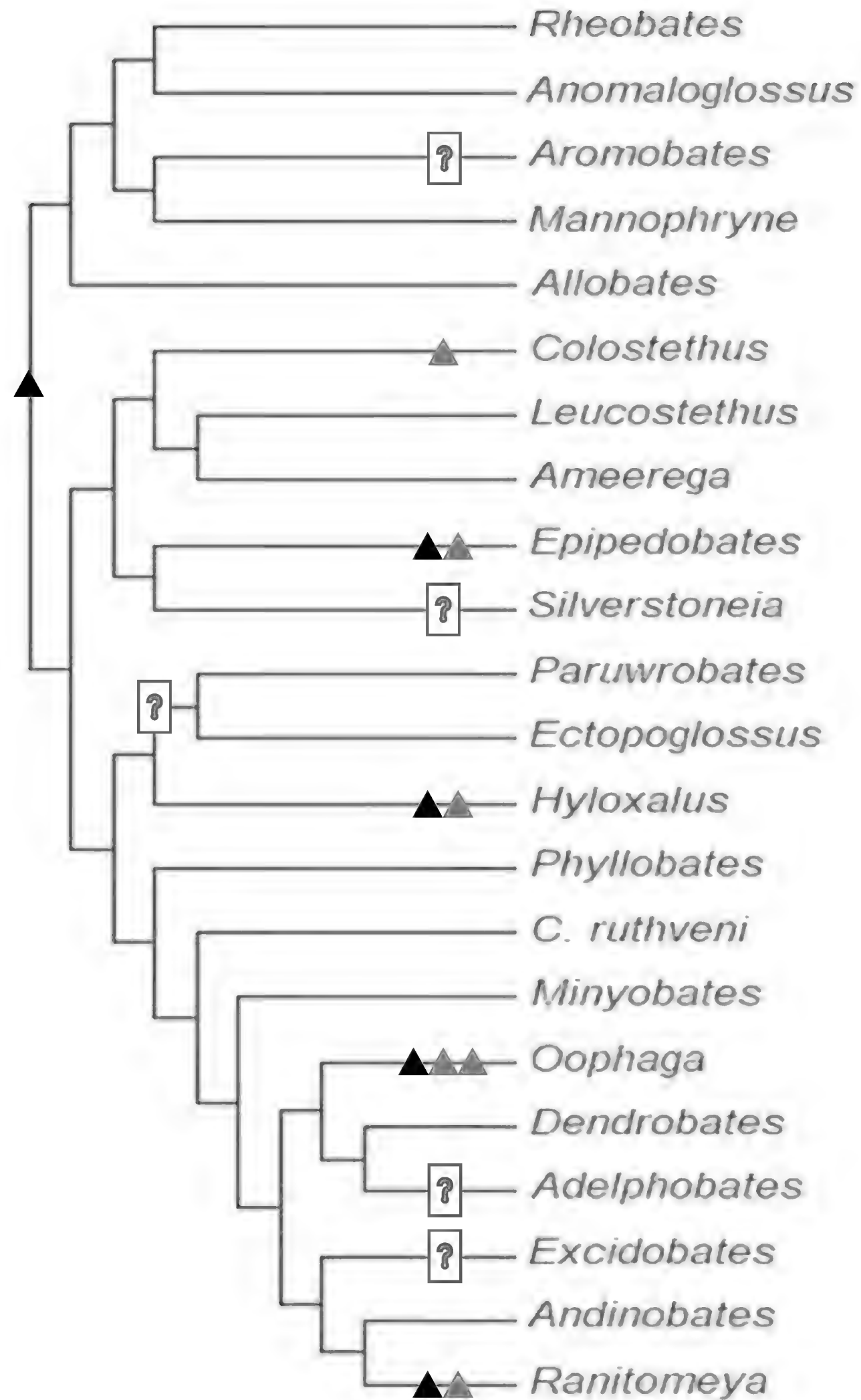
- ▲ 0. aquatic
- ▲ 1. terrestrial: leaf litter, soil, on or under stones
- ▲ 2. terrestrial: phytotelmata
- ▲ 3. terrestrial: on top or underside of leaves above ground or water level.



119. Egg clutch attendance occurrence

▲ 0. absent

▲ 1. present

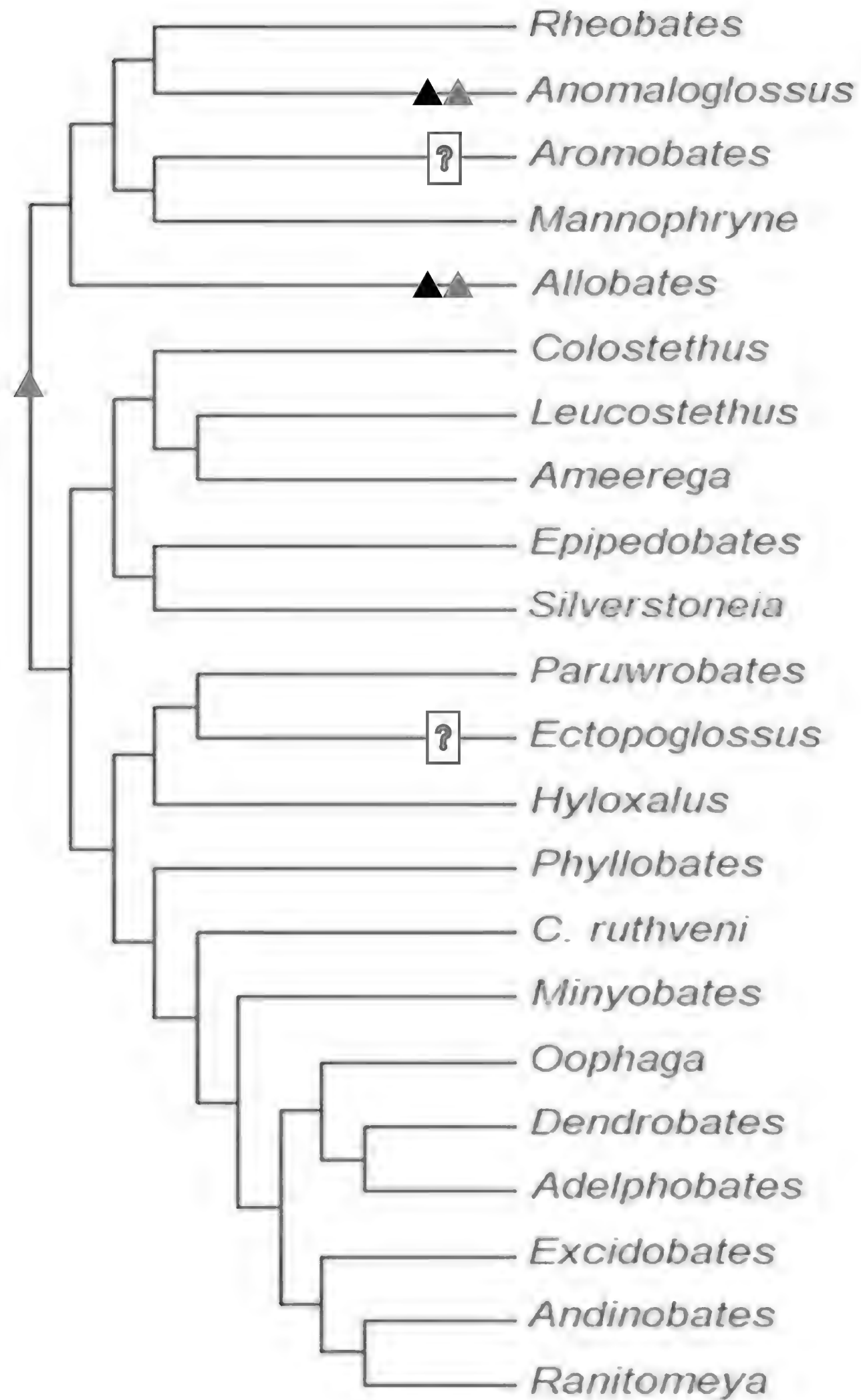


120. Egg clutch attendant sex

▲ 0. male

▲ 1. female

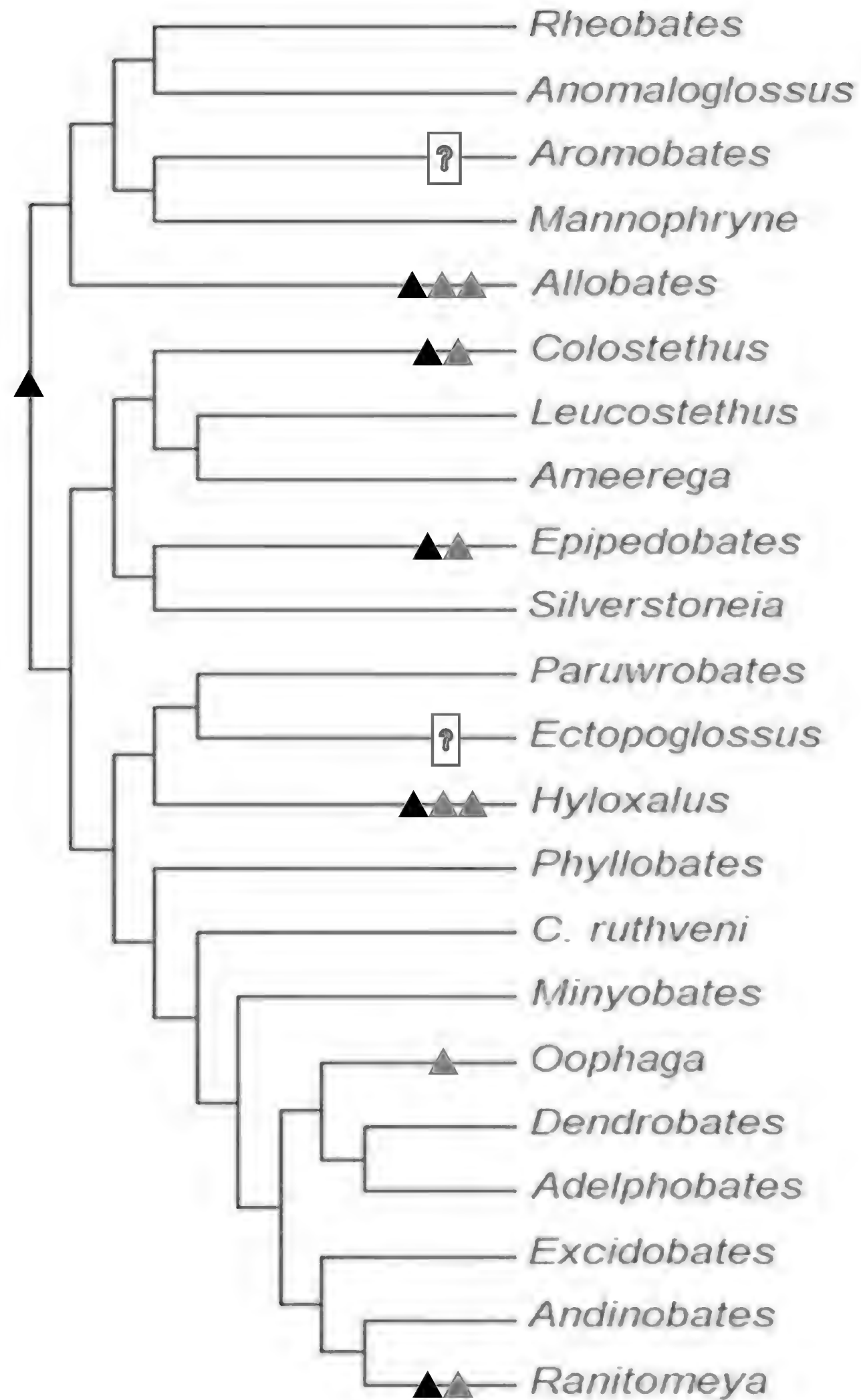
▲ 2. both



121. Dorsal tadpole transport

▲ 0. absent

▲ 1. present



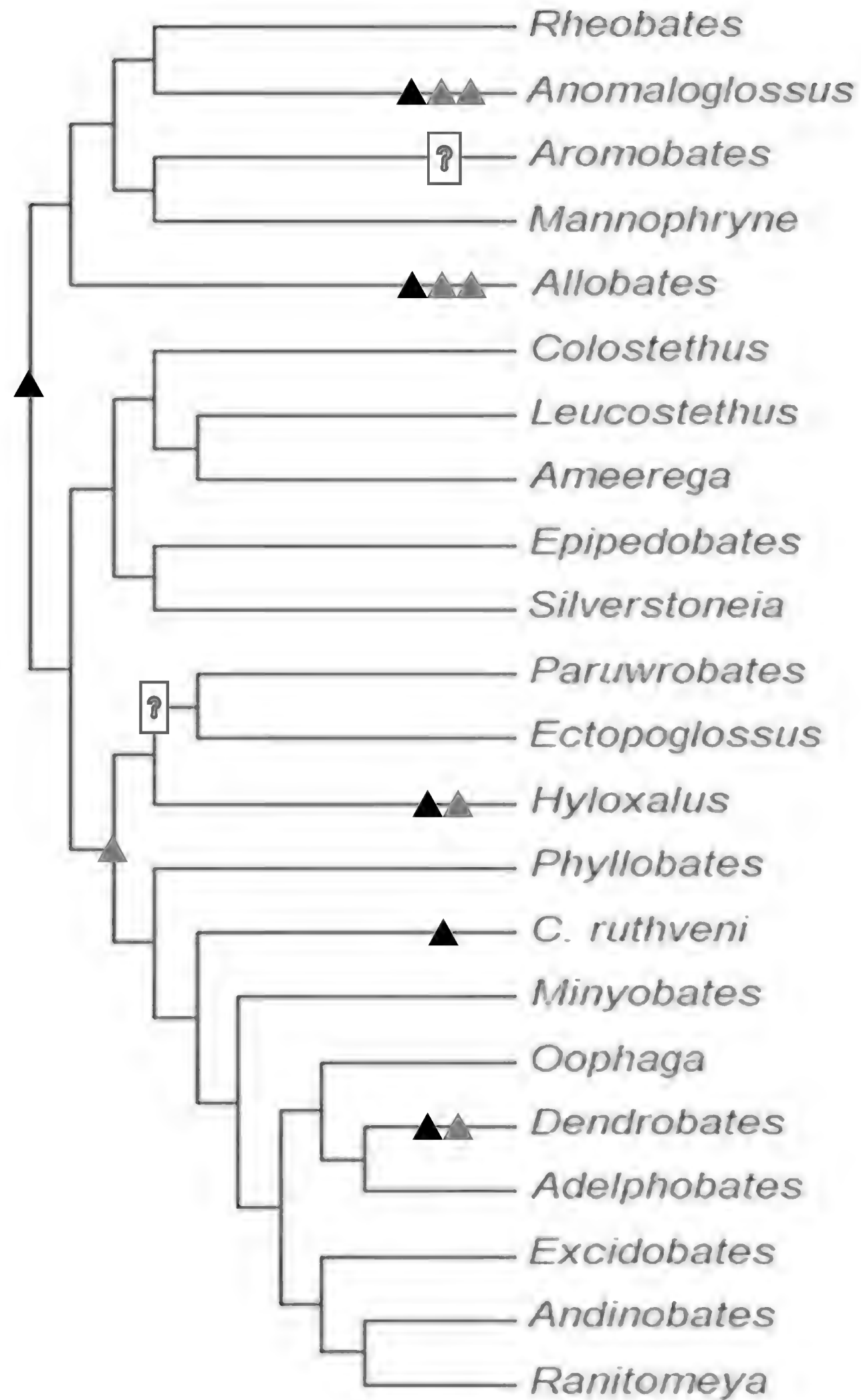
122. Sex of nurse frog

▲ 0. male

▲ 1. female

▲ 2. both

Nonadditive.



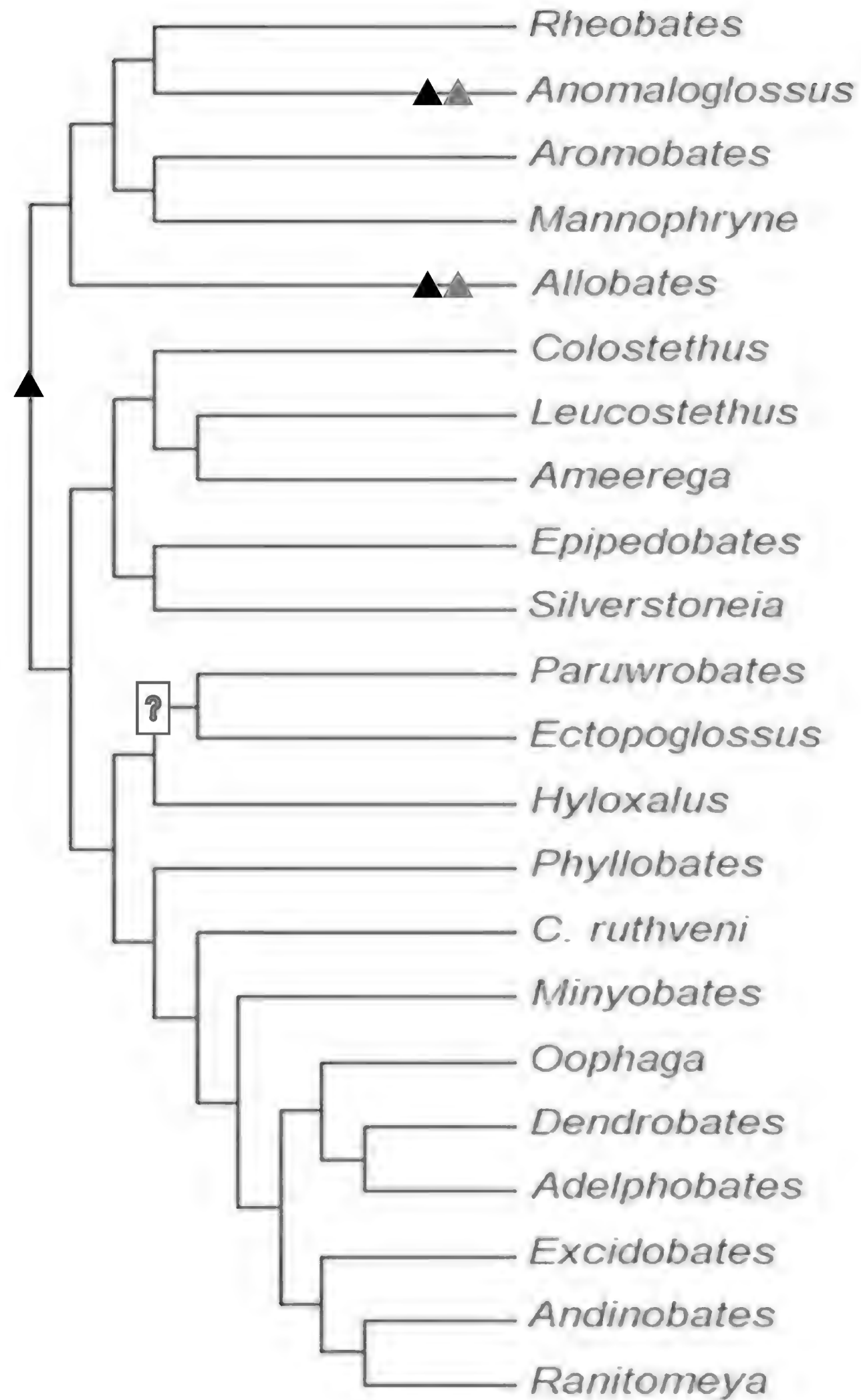
123. Larval habitat

▲ 0. pool or stream

▲ 1. phytotelmata

▲ 2. nidicolous

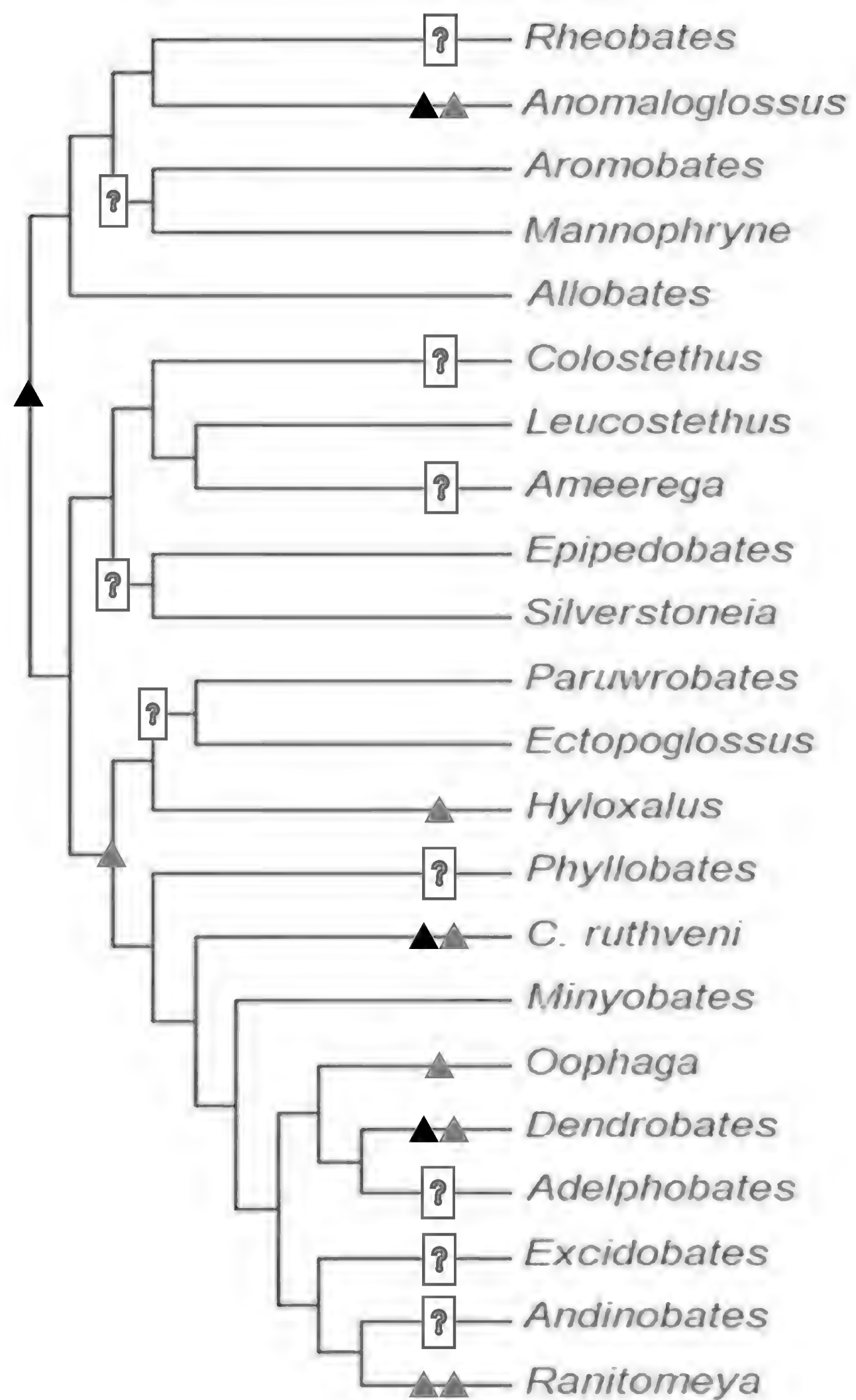
Nonadditive.



124. Larval trophic guild

▲ 0. exotrophic

▲ 1. endrotrophic



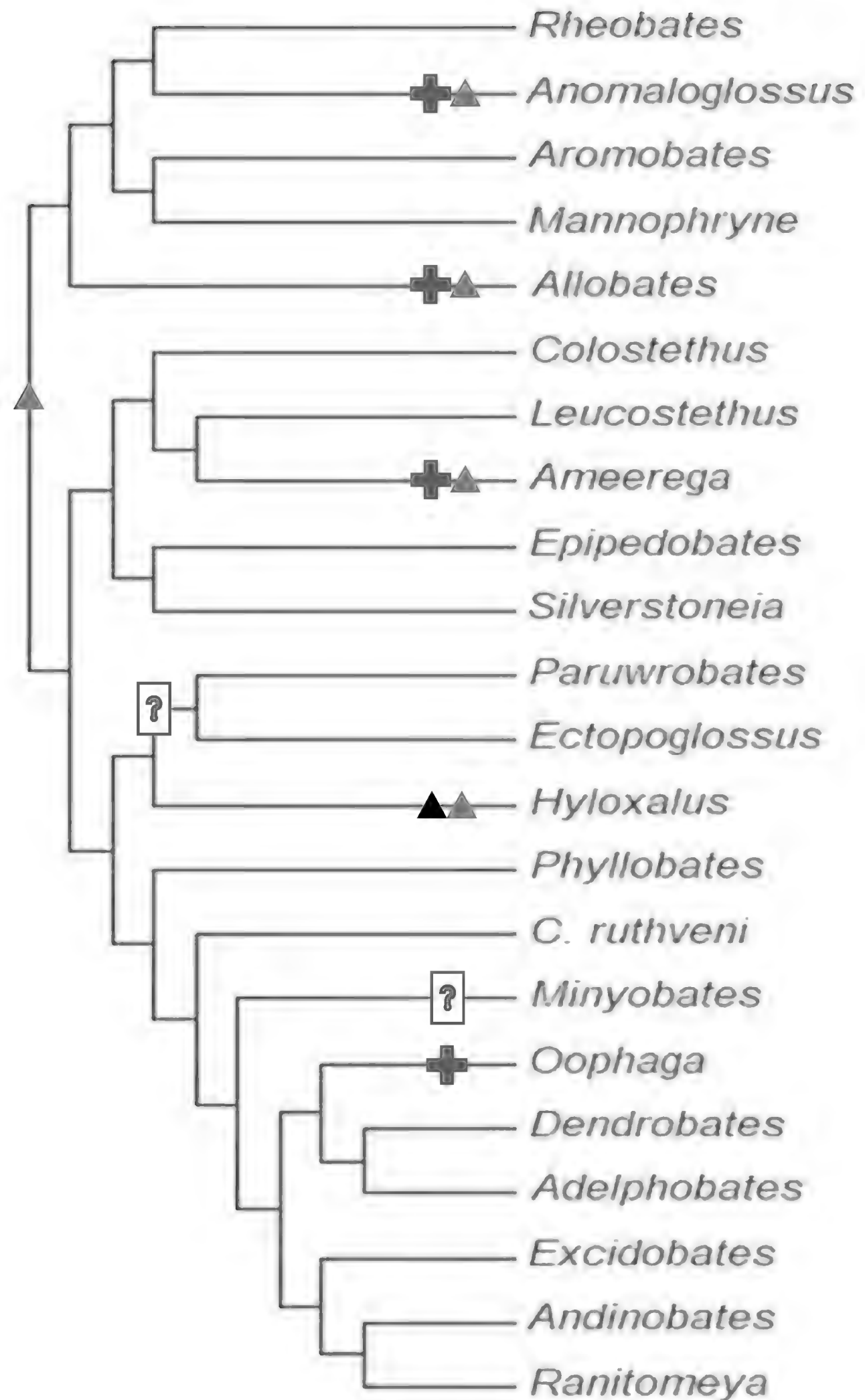
125. Exotrophic larval diet

▲ 0. detritivorous

▲ 1. predaceous

▲ 2. oophagus

Nonadditive.

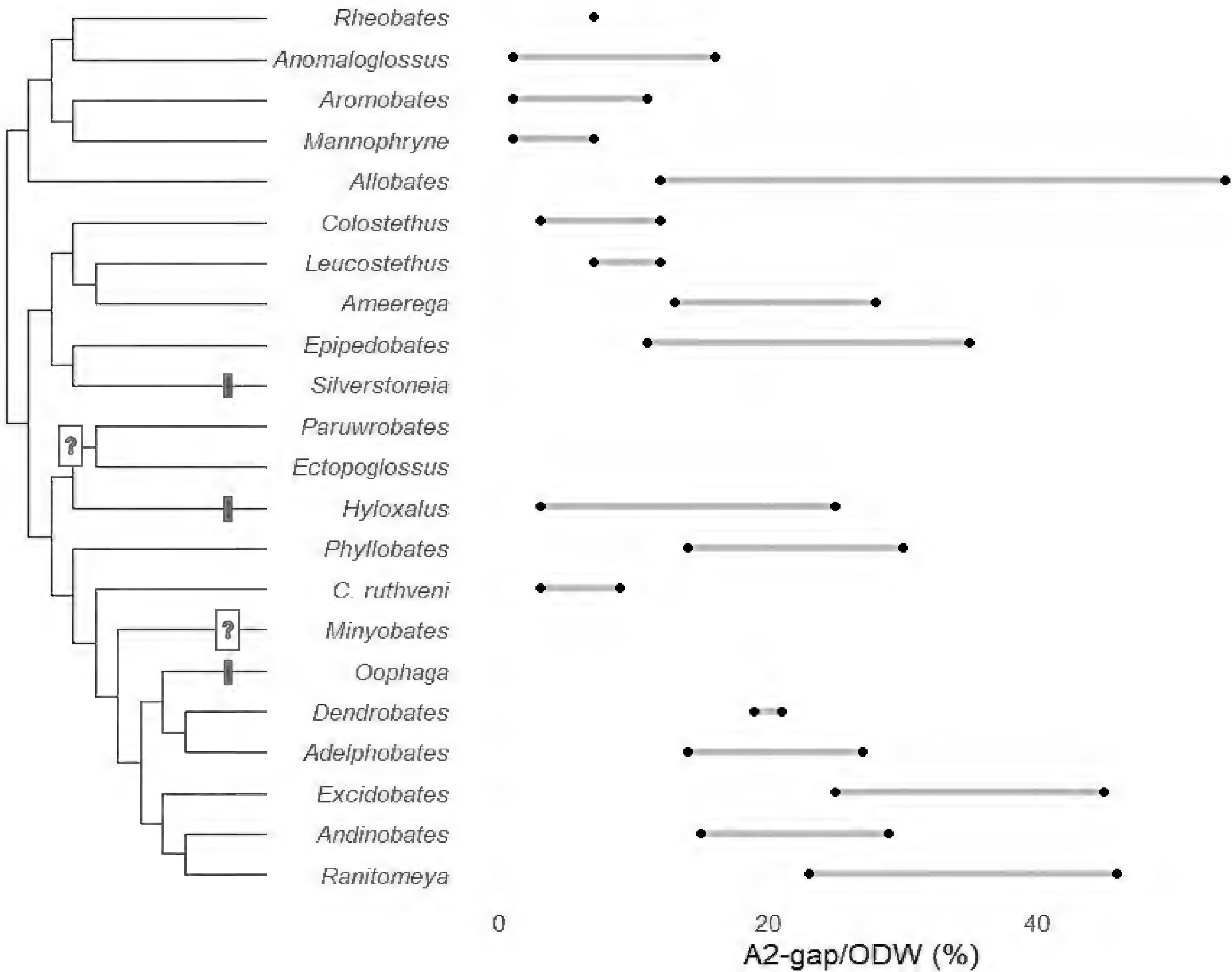


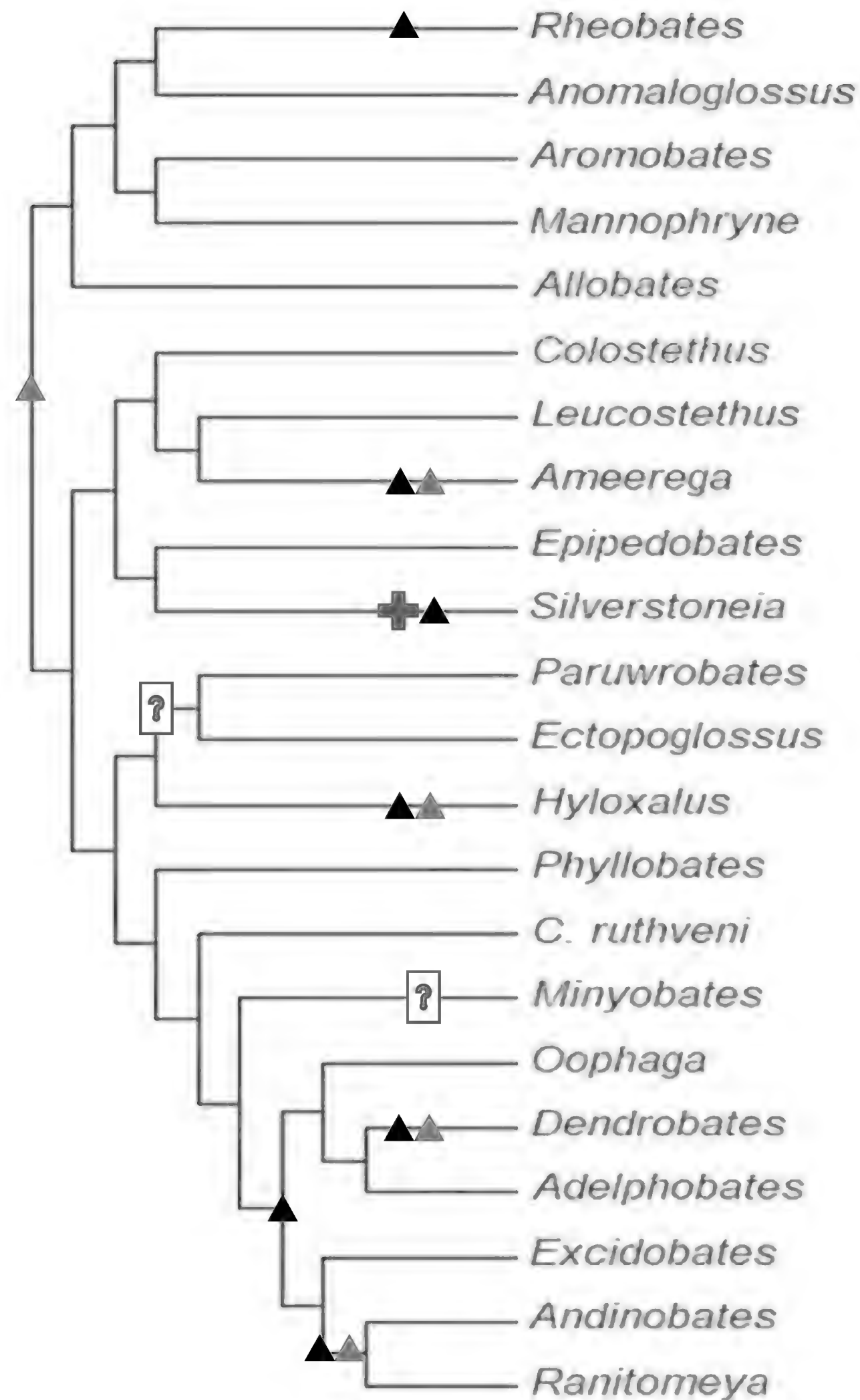
189. Second row of keratodont tooth of anterior lip, medial gap (A2-gap)

▲ 0. absent

▲ 1. present

⊕ NA





190. First row of keratodont tooth of posterior lip, medial gap (P1-gap)

▲ 0. absent

▲ 1. present

⊕ NA



Reptiles of the King Abdulaziz Royal Nature Reserve, east-central Saudi Arabia: insights and conservation implications

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Abstract.—The distribution of herpetofauna on the Arabian Peninsula is generally poorly known, particularly in Saudi Arabia. The King Abdulaziz Royal Nature Reserve (KARNR) in east-central Saudi Arabia is in an area predicted to have high reptile species richness, yet there is no baseline reptile checklist for the reserve. Knowing which species occur within a protected area is vital for ensuring that conservation strategies and long-term monitoring are effective. Here, we provide the first detailed list of reptile fauna occurring in the KARNR. This study recorded a total of 31 species from 1,551 observations, including 25 lizards and six snakes. A desktop study identified three additional species, bringing the total number of herpetofaunal species known in the KARNR to 34. Two of the species found in the reserve are of conservation concern: *Uromastix aegyptia* (VU) and *Tropicolotes wolfgangboehmei* (DD). Through species accumulation curves we were able to determine that the sampling methods implemented during the survey were adequate, and we predicted that the species richness of the reserve is likely as much as 38 species. Prior to this study, only 82 reptile records (30 species) were known from the study area, so we add a significant number of new records to the reserve and an additional four species that were previously unknown from the region.

Keywords. Arabian Peninsula, baseline, iNEXT, protected area, species accumulation curves, species richness, Squamata, *Tropicolotes wolfgangboehmei*, *Uromastix aegyptia*

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Introduction

Reptile species richness on the Arabian Peninsula is highest in the mountainous regions along the south-western, southern, and eastern parts of the peninsula (Carranza et al. 2021; Šmíd et al. 2021). However, the central desert regions also have relatively high species richness and phylogenetic diversity (Cox et al. 2012; Šmíd et al. 2021; Tamar et al. 2023). The Kingdom of Saudi Arabia, which covers most of the Arabian Peninsula, is well known for its large expansive deserts and a number of regional inventories of reptiles have been compiled over the past few decades (i.e., Al Sadoon et al. 2016, 2017; Aloufi et al. 2021, 2022; Alshammari and Busais 2020; Alshammari et al. 2017; Alshammari and Ibrahim 2015; Aloufi and Amr 2015; Cunningham 2010; Farag and Banaja 1980; Tilbury 1998). Despite these inventories, large areas of Saudi Arabia are poorly

surveyed for reptiles at the landscape scale, so our current knowledge of the distribution and community composition of Saudi Arabia's reptiles is inadequate (Šmíd et al. 2021).

The King Abdulaziz Royal Nature Reserve (KARNR) in east-central Saudi Arabia was established in 2018 and is one of seven royal reserves in the country (<https://www.karnr.gov.sa/>). The reserve covers a heterogeneous landscape of just over 28,000 km², and this region is predicted to have high terrestrial reptile species richness (Alatawi et al. 2020; Ficetola et al. 2013; Šmíd et al. 2021). However, there have been no dedicated surveys on the reptile species richness of the area. The lack of such inventories in protected areas is detrimental to reptile conservation as there are no baseline data to inform conservation strategies and long-term monitoring methods in the reserve (Margules and Pressey 2000; Mihoub 2017).

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This study aimed to provide an adequate baseline of reptile species richness in the KARNR. To achieve this, we conducted field work on five occasions between November 2022 and October 2023 for a total of 46 days, which equated to 139 person-days in the field. A desktop study was also conducted to identify any reptile species previously recorded from the area. We tested the adequacy of our survey effort using species accumulation curves and estimated diversity indices.

Materials and Methods

Study Area

The KARNR is situated in east-central Saudi Arabia. It is located between the northern Riyadh region and northwestern Eastern Province and covers an area of just over 28,000 km² (Fig. 1). Our study area was the KARNR plus a 10 km buffer around the boundary of the reserve. According to the Köppen-Geiger classification, this region is categorized as a hot desert (Peel et al. 2007). The major geomorphological features of the study area are the Al Summan plateau in the north of the reserve, the Ad Dahna sand desert that crosses the reserve nearly transversely in the middle, and the Tuwaiq Escarpment, a mountain ridge that cuts through the southern part of the reserve (Almalki et al. 2022; Vincent 2008). The influence of these sandy and rocky formations has resulted in a high habitat heterogeneity for the region, with rocky areas (such as escarpments and rock outcrops), open plains (stoney and gravel plains), dunes (mobile and vegetated dunes), and wetland areas and drainage lines (wadis and rawdhats) (Fig. 2). A general north/south moisture gradient is evident from the vegetation and plant communities in the study area, with

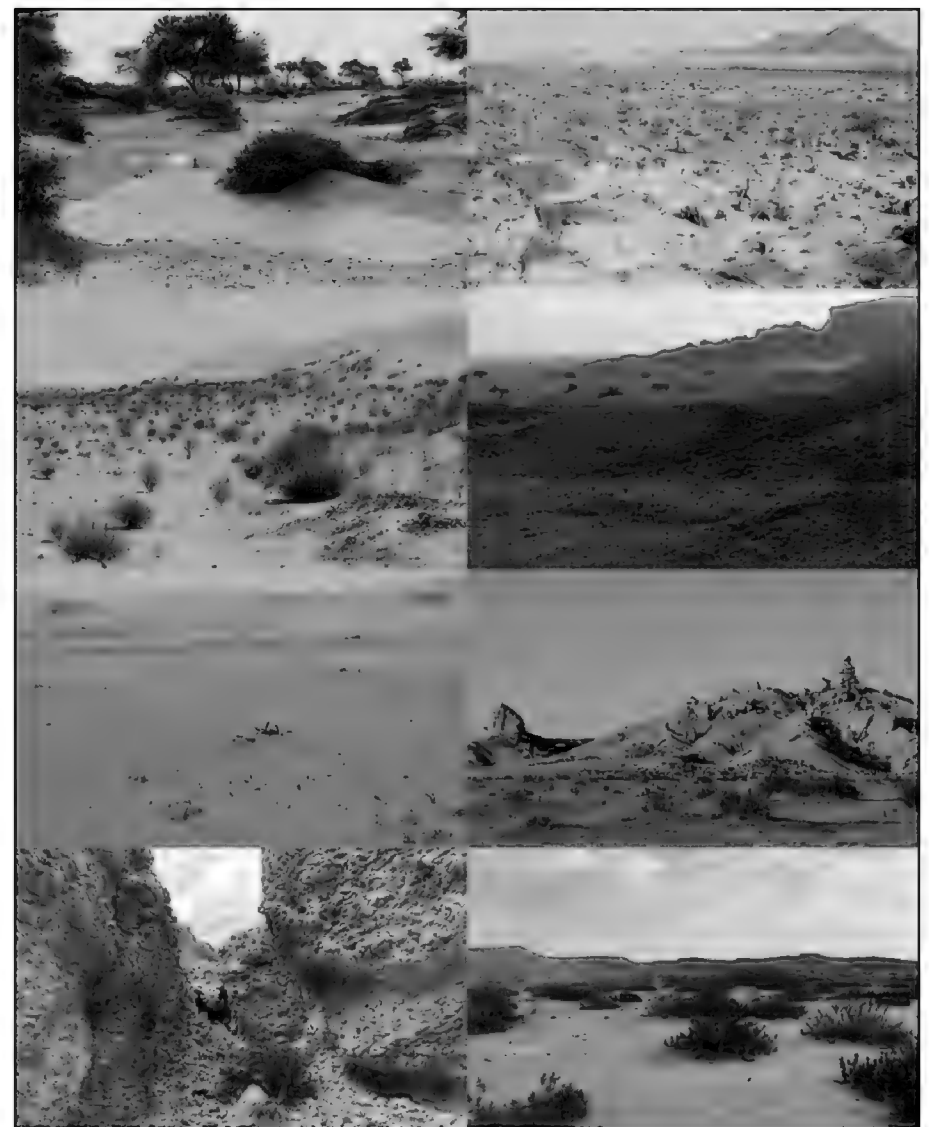


Fig. 2. Several landscape features of the King Abdulaziz Royal Nature Reserve, showing some of the habitat diversity in the region.

the southern areas having more trees and rawdhat areas (Ghazanfar and Fisher 1998).

Methods

Field surveys. Three primary field surveys were carried out for 21 days from 23 November to 13 December 2022, 18 days from 3 to 20 March 2023, and eight days from 28

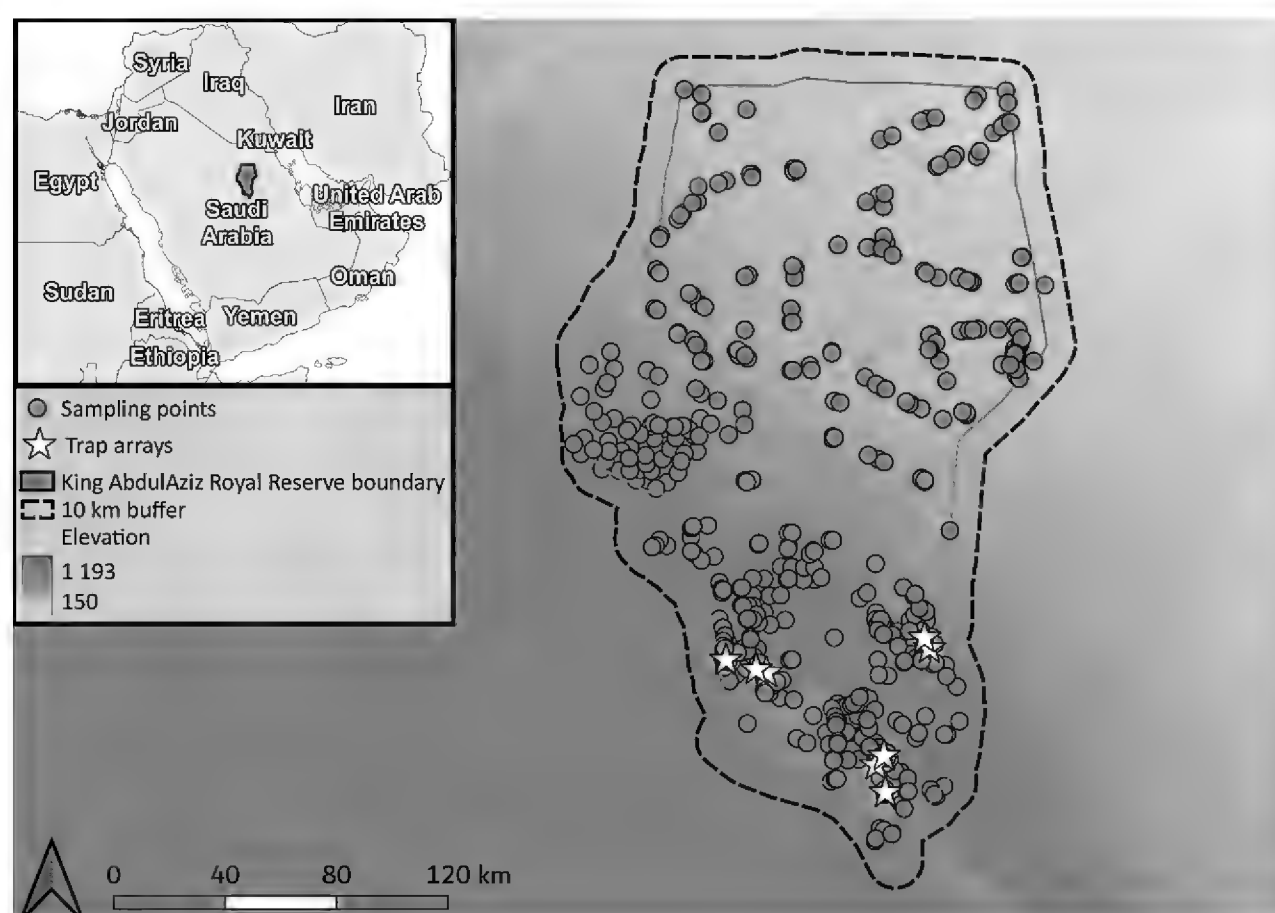


Fig. 1. The King Abdulaziz Royal Nature Reserve study area and its location in the Arabian Peninsula. Blue dots show sampling points visited during the 2022/23 survey, and white stars show reptile trap array locations.

September to 5 October 2023. Two opportunistic surveys were also conducted for a five-day period, which formed part of larger scale rapid biodiversity overviews and were more limited in effort than the three primary surveys. One was from 17 to 21 February 2023 and another was from 8 to 12 June 2023. Collectively, these covered a total of 57 survey-days and 139 person-days. Sampling methods during the field surveys included active searching (both diurnal and nocturnal, see below) and trapping (6 March until 19 March 2023). Observations recorded by team members surveying other taxa were also included as supplementary records. The February 2023 opportunistic survey also targeted wetland areas after a good rain season to check for the occurrence of amphibians in the study area. The June 2023 opportunistic survey was conducted during a hot period of the year and had a specific focus on nocturnal active searches. Reptiles were recorded to the species level when possible. Occasionally some individuals could only be identified to genus when species assignment was uncertain. When feasible, DNA barcoding was conducted to aid in the identification in these instances. For each record, a GPS location (with an average accuracy of 5 m) was taken using Garmin Montana i750 GPS units, and specimen abundance was noted.

Active searching was conducted at 374 specific point locations across the reserve (Fig. 1). These points were selected by overlaying a 1 km sampling grid across the study area and using a multistage, stratified, semi-random sampling approach. The northern section of the reserve, Al Summan, was only surveyed during November–December 2022 and September 2023 due to logistical constraints. The southern section of the reserve was surveyed during all sampling seasons. Diurnal surveys were conducted between 0600 and 1900 h, while nocturnal surveys took place between 1900 and 2300 h.

Eight trap arrays were deployed within the study area. The placement of trap arrays was limited to areas near main roads due to logistical convenience. Each trap array consisted of three 10 m drift fences of robust plastic, arranged in a linear design as described by Mendes et al. (2015), together with four 20 L buckets sunk into the ground as pitfall traps, and six wire-mesh funnel traps positioned on each side of the drift fence between the pitfall traps (Fig. 3). The funnel traps were covered with shade netting to provide shelter for captured animals, while moistened leaf-litter and cloth were added to the buckets to provide shelter and moisture in the pitfall traps. These traps were checked daily for captured animals. The eight trap arrays were installed for 13 nights, equating to 104 trap array-nights.

A desktop survey was also conducted to consolidate historical reptile records for the study area. This was achieved by consulting various literature sources (e.g., Aloufi et al. 2019; Al-Sadoon 1988; Al-Sadoon 1989; Gasperetti 1988; Kordges 1998; Salvador 1982; Šmíd et al. 2021) and online databases (e.g., Global Biodiversity

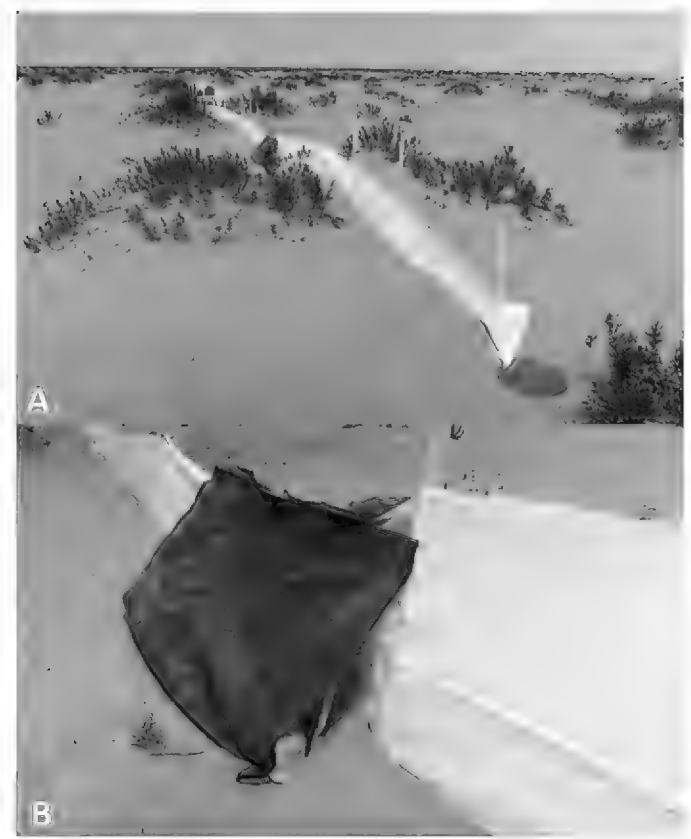


Fig. 3. (A) An example of a trap array showing drift fence and pitfall trap. (B) Detail of a funnel trap with shade netting cover.

Information Facility at <http://GBIF.org> and iNaturalist at <http://inaturalist.org>). All photographic records obtained during the 2022/23 survey were uploaded to iNaturalist and these are available in Supplemental Table S1.

DNA sequencing. Some species of Arabian reptiles are challenging to identify based on morphological characters alone. We therefore sequenced a short fragment of one mitochondrial gene and applied DNA barcoding methods to verify the identification in the field. Of the species recorded during the surveys, we DNA barcoded members of the following genera: *Acanthodactylus* and *Mesalina* (both Lacertidae); *Pseudotrapelus* and *Trapelus* (both Agamidae), *Hemidactylus* and *Stenodactylus* (both Gekkonidae), *Scincus* (Scincidae), and *Ptyodactylus* (Phyllodactylidae). Tissue samples were obtained by taking a small piece of tail. All samples were preserved in 96% ethanol and DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. We PCR-amplified and sequenced the ribosomal 12S rRNA (12S) region of the mitochondrial DNA for all groups except the agamids, for which we sequenced the 16S rRNA (16S). Primers and PCR conditions are detailed in Šmíd et al. (2021). The PCR products were sequenced from both directions at Macrogen (Amsterdam, the Netherlands), and raw sequence data were edited and assembled in Geneious v.11 (Kearse et al. 2012). The identification of each specimen was confirmed using the BLAST algorithm of the National Center for Biotechnology Information website (<https://blast.ncbi.nlm.nih.gov/>).

Species Accumulation Curves

Total survey. To assess our survey effort in the KARNR, we used species accumulation curves calculated in R (R

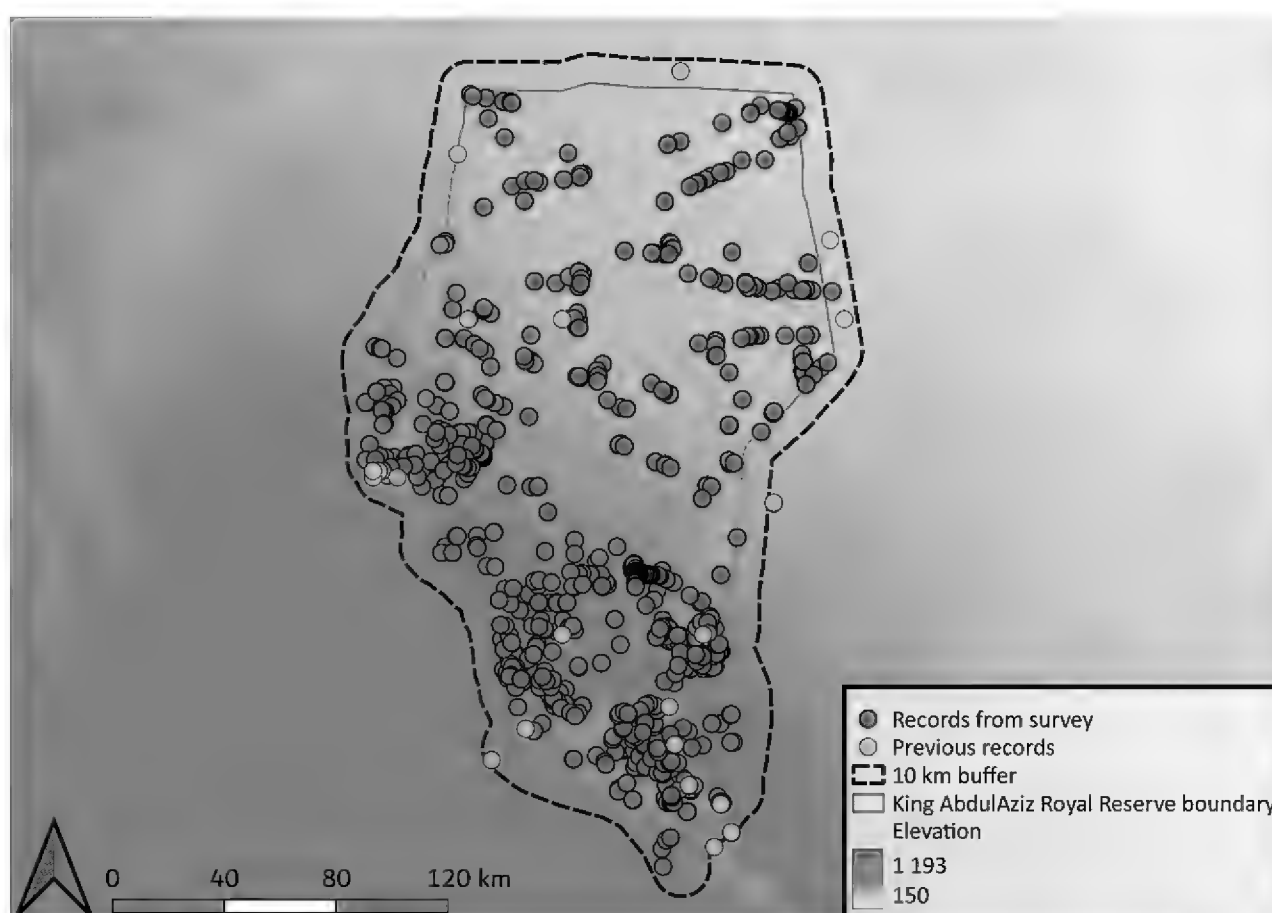


Fig. 4. Records of reptiles found during the 2022/23 survey period (purple circles) and from the desktop study (blue circles) in the King Abdulaziz Royal Nature Reserve.

Core Team 2022) using the R studio console (RStudio team 2022) with the iNEXT package (Hsieh et al. 2016). Species accumulation curves were plotted and extrapolated to double the original survey effort (Hsieh et al. 2016), and the order of the observations made during the survey were randomized 100 times in a bootstrap fashion. The estimated species richness was calculated in iNEXT along with the standard error and lower and upper confidence levels. Shannon and Simpson diversity indices were also calculated. Four broad-scale reptile habitat types were identified for the KARNR: (1) Dunes and dune valleys, (2) Hills, outcrops, and escarpments, (3) Plains, and (4) Wadis, rawhdats, and pans. These habitat types were defined based on vegetation and topographic characteristics. For each habitat type, predictive species accumulation curves were provided to assess the sampling effort for each of the broad habitat types.

The survey species richness was then evaluated in terms of completeness according to the iNEXT estimated species richness. A percentage of the completeness score was calculated as $\text{SurveySR} / \text{iNEXT Estimated SR} \times 100$. Any result greater than 90% is regarded as “close to completely surveyed,” since it can be unlikely that all species could be recorded during a finite survey (Chao and Jost 2012; Moreno and Halffter 2001; Thompson et al. 2007). Note that the value calculated is an estimate based on the species accumulation curve, and therefore dependent on the sampling effort.

Survey effort was also assessed for each habitat type to evaluate diversity at the broad-scale habitat level. For this, the vegetation class for each species record was extracted from a vegetation layer generated for the suite of studies conducted for the present project (Van Rooyen et al. 2023) using QGIS 3.22.8 (QGIS Development Team 2023). Each vegetation type class represented in

the reserve was then grouped into broad habitat units of Dunes and dune valleys; Plains; Outcrops, hills and escarpments; and Wadis, rawhdats and pans. The methods above were used to create species accumulation curves and estimate species richness.

Results

A total of 1,551 reptile observations of 31 species were made during the 2022/2023 survey period, composed of 25 lizards and six snakes (Table 1, Supplemental Table S1, Figs. 4–9). Four species had not been previously recorded in the area: *Trapelus agnetae*, *Hemidactylus granosus*, *Mesalina saudiarabica*, and *Diplometopon zarudnyi*. The desktop study of published and online sources produced 82 records of 30 species, three of which were not recorded during our survey: *Platycephalus rhodorachis*, *Myriopholis macrorhyncha*, and *Walterinnesia morgani*. Therefore, the total known reptile species richness for the KARNR is currently 34 species.

Most reptile observations were recorded via active searching ($n = 1,400$). Fifty-three individuals were captured by trap arrays, with the most common being *Acanthodactylus opheodurus* ($n = 11$). The remaining 91 records were all incidental. The most common species encountered was *Bunopus tuberculatus* ($n = 579$), followed by *Uromastix aegyptia* ($n = 367$). Two species were recorded only once during the survey: *Acanthodactylus hardyi* and *Echis coloratus* (Table 1).

Two species of conservation concern were found within the reserve borders: *U. aegyptia* (Vulnerable) and *Tropicolotes wolfgangboehmei* (Gekkonidae; Data Deficient) (IUCN 2023). Although *U. aegyptia* was very common throughout the reserve, only two *T. wolfgangboehmei* were found. One of the localities for *T.*

Table 1. Checklist and abundance of reptile species recorded in the King AbdulAziz Royal Nature Reserve. The International Union for Conservation of Nature (IUCN 2023) listings of conservation status refer to the following categories: Not Evaluated (NE), Least Concern (LC), Data Deficient (DD), Vulnerable (VU), and Endangered (EN).

Scientific name	Common name	IUCN status	Number of observations	Recorded during survey	Recorded prior to survey
SAURIA		Lizards			
Family Agamidae					
<i>Phrynocephalus arabicus</i>	Arabian Toad-headed Agama	LC	26	Y	Y
<i>Pseudotrapelus tuwaiqensis</i>	Tuwaiq Agama	NE	2	Y	Y
<i>Trapelus agnetae</i>	Northern Arabian Plain Agama	LC	4	Y	N
<i>Trapelus ruderatus</i>	Olivier’s Agama	LC	4	Y	Y
<i>Uromastyx aegyptia</i>	Egyptian Spiny-tailed Lizard	VU	367	Y	Y
Family Gekkonidae					
<i>Bunopus tuberculatus</i>	Baluch Ground Gecko	LC	579	Y	Y
<i>Cyrtopodion scabrum</i>	Rough-tailed Gecko	LC	4	Y	Y
<i>Hemidactylus granosus</i>	West Arabian Half-toed Gecko	NE	5	Y	N
<i>Stenodactylus doriae</i>	Middle Eastern Short-fingered Gecko	LC	44	Y	Y
<i>Stenodactylus slevini</i>	Slevin’s Short-fingered Gecko	LC	16	Y	Y
<i>Trigonodactylus arabicus</i>	Arabian Web-footed Sand Gecko	LC	13	Y	Y
<i>Tropicolotes wolfgangboehmei</i>	Wolfgang’s Sand Gecko	DD	2	Y	Y
Family Phyllodactylidae					
<i>Ptyodactylus cf. hasselquistii</i>	Hasselquist’s Fan-footed Gecko	LC	107	Y	Y
Family Lacertidae					
<i>Acanthodactylus boskianus</i>	Bosc’s Fringe-fingered Lizard	LC	24	Y	Y
<i>Acanthodactylus hardyi</i>	Hardy’s Fringe-fingered Lizard	NE	1	Y	Y
<i>Acanthodactylus opheodurus</i>	Snake-tailed Fringe-fingered Lizard	LC	54	Y	Y
<i>Acanthodactylus schmidtii</i>	Schmidt’s Fringe-fingered Lizard	LC	57	Y	Y
<i>Mesalina brevirostris</i>	Short-nosed Desert Lizard	LC	102	Y	Y
<i>Mesalina</i> lineage 4	Small Spotted Desert Racer	-	46	Y	Y
<i>Mesalina saudiarabica</i>	Arabian Short-nosed Desert Lizard	NE	3	Y	N
Family Scincidae					
<i>Chalcides ocellatus</i>	Ocellated Skink	LC	8	Y	Y
<i>Scincus mitranus</i>	Arabian Sandfish	LC	12	Y	Y
<i>Scincus conirostris</i>	Sandfish Skink	NE	12	Y	Y
Family Trogonophidae					
<i>Diplometopon zarudnyi</i>	Zarudny’s Worm Lizard	LC	23	Y	N
Family Varanidae					
<i>Varanus griseus</i>	Desert Monitor	LC	7	Y	Y

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SERPENTES	Snakes				
Family Boidae					
<i>Eryx jayakari</i>	Arabian Sand Boa	LC	2	Y	Y
Family Colubridae					
<i>Platyceps rhodorachis</i>	Braid Snake	LC	0	N	Y
<i>Spalerosophis diadema</i>	Diadem Snake	LC	5	Y	Y
Family Elapidae					
<i>Walterinnesia morgani</i>	Black Desert Cobra	LC	0	N	Y
Family Leptotyphlopidae					
<i>Myriopholis macrorhyncha</i>	Beaked Thread Snake	LC	0	N	Y
Family Psammophiidae					
<i>Malpolon moilensis</i>	Hooded Malpolon	LC	6	Y	Y
<i>Psammophis schokari</i>	Afro-Asian Sand Snake	LC	4	Y	Y
Family Viperidae					
<i>Cerastes gasperettii</i>	Arabian Horned Viper	LC	11	Y	Y
<i>Echis coloratus</i>	Palestine Saw-scaled Viper	LC	1	Y	Y
Total observations and species counts			1,551	31	30
Total species for KARNR				34	

wolfgangboehmei was inside the 10 km buffer zone from a known site, whereas the other was inside the reserve and represents a new locality record for this species.

DNA Barcoding

We DNA barcoded a total of 95 samples in the families Agamidae (four samples), Gekkonidae (13 samples), Lacertidae (59 samples), Phyllodactylidae (seven samples), and Scincidae (12 samples). The results mostly confirmed our field identifications. However, two species were misidentified in the field and were only revealed by the DNA analysis. These were one specimen of *Acanthodactylus hardyi* and three specimens of *Mesalina saudiarabica*, both from the vicinity of the Ad Dahna sand stretch. It is important to note the majority of *Mesalina brevirostris* specimens were not DNA barcoded, so some of these may be incorrectly identified as *M. saudiarabica*. Details on the DNA barcoding results along with GenBank accessions of the newly sequenced samples are given in Supplemental Table S2.

Species Accumulation Curves

The predictive species accumulation curve indicates that the sampling methods employed during the field surveys were adequate, with the curve reaching an asymptote (Fig. 10) and an estimated 97.89% of reptile species

being recorded, based on the iNEXT completeness score (Table 2). The species accumulation curve predicts that the species richness for the KARNR is between 31 and 38 species (LCL = 31; UCL = 37.84; Table 2).

Dunes and dune valleys are the least well sampled of the broad habitat units, with the accumulation curve not reaching an asymptote (Fig. 11) and an estimated 63.75% of the reptile species being recorded based on the iNEXT completeness score (Table 2). Plains are relatively well sampled, but the iNEXT completeness score of 88.75% suggests that additional species are likely to be found in this area, with an estimated species richness between 21 and 38 (LCL = 21.0; UCL = 38.05). Wadis, rawhdats, and pans also appear to be well sampled with an iNEXT completeness of 94.93%. Hills, outcrops, and escarpments are the most well sampled habitat type with iNEXT completeness scores of over 98% and accumulation curves approaching asymptotes (Table 2; Fig. 11).

Discussion

There are now 34 species of reptiles confirmed to occur within the KARNR. Thirty-one of these species were found during our survey, four of which had not previously been recorded in this area. This study contributes a substantial number of observations for the region, with 1,550 individual records across the reserve, compared

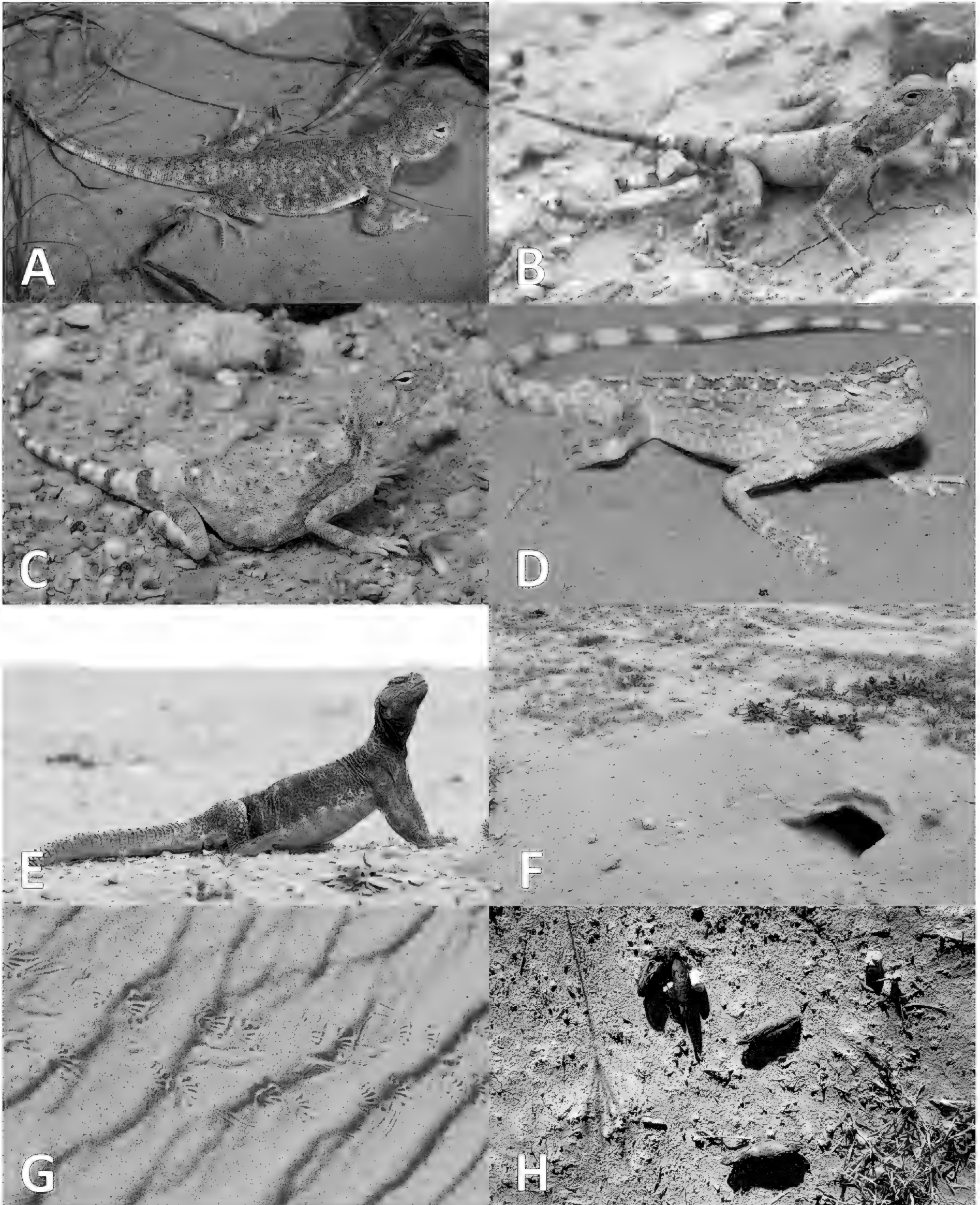


Fig. 5. (A) *Phrynocephalus arabicus*, (B) *Pseudotrapelus tuwaiqensis*, (C) *Trapelus agnetae*, (D) *Trapelus ruderatus*, (E) *Uromastix aegyptia*, (F) *Uromastix aegyptia* burrow, (G) *Uromastix aegyptia* tracks, (H) *Uromastix aegyptia* scat.

to only 82 known prior to our survey. The number of species recorded in the reserve is expected to increase as taxonomic revisions are completed and as further surveys are conducted. The information generated here will aid the conservation efforts of the reserve, provide

a baseline for long-term monitoring, and inform further regional research and conservation assessments.

The 1,551 records generated for the reserve add a substantial number of observations not only for KARNR, but also for the whole of the Arabian Peninsula, with an

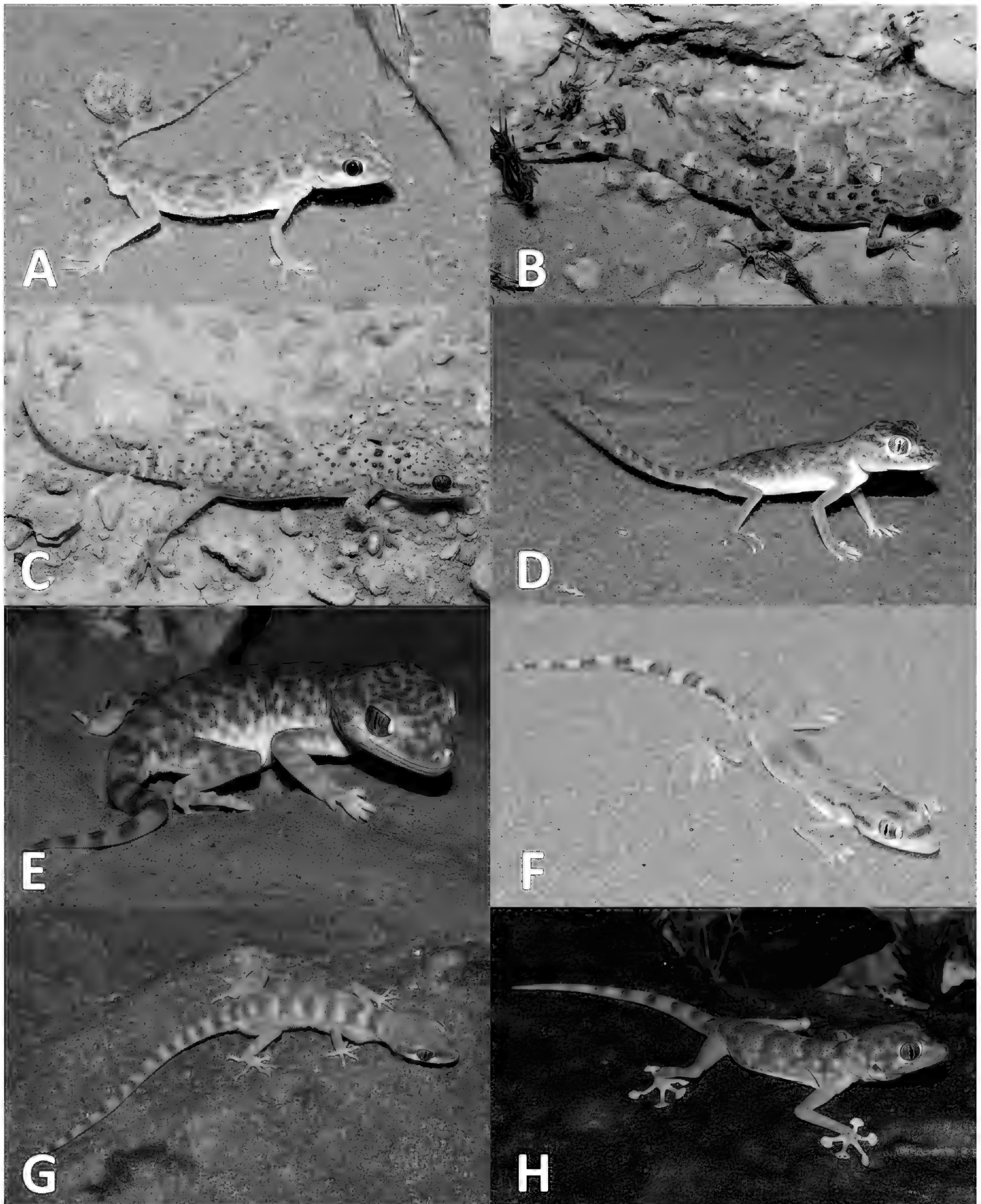


Fig. 6. (A) *Bunopus tuberculatus*, (B) *Cyrtopodion scabrum*, (C) *Hemidactylus granosus*, (D) *Stenodactylus doriae*, (E) *Stenodactylus slevini*, (F) *Trigonodactylus arabicus*, (G) *Tropicolotes wolfgangboehmei*, (H) *Ptyodactylus* cf. *hasselquistii*.

8.59% increase based on 18,053 records for the region as of 2021 (Šmíd et al. 2021). Having a baseline of reptile species diversity (richness and abundance) is important from a conservation perspective, as the data can be used to monitor changes over time (Maritz et al. 2016). Without such diversity metrics, the decay of richness

and abundance, potentially signalling local extinction events (Cressey et al. 2015; Zipkin et al. 2020), will go undetected and the “shifting baseline” syndrome is likely to take effect (Pauly 1995; Soga and Gaston 2018). For KARNR, this established baseline places conservation managers and stakeholders in a position to monitor future

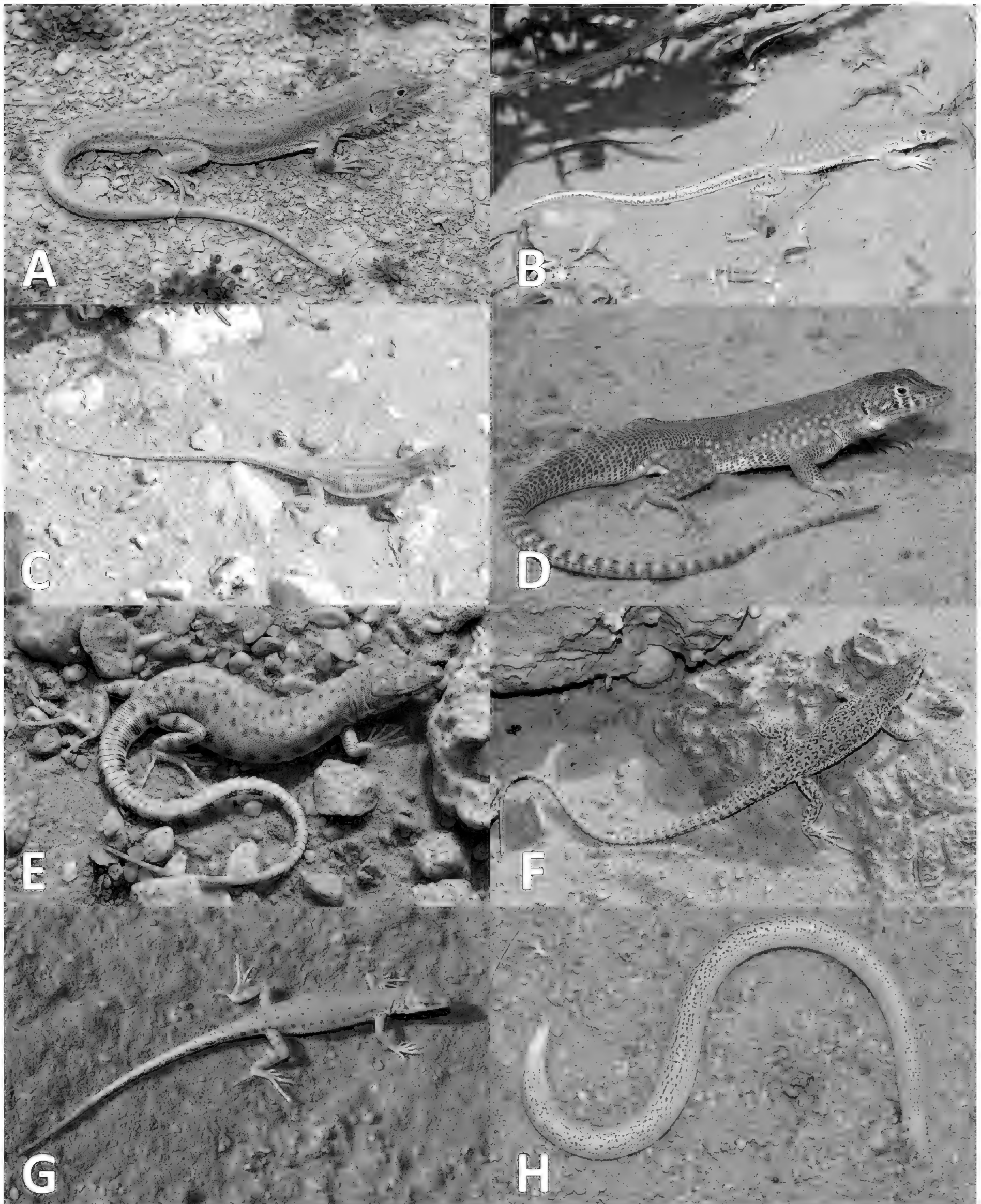


Fig. 7. (A) *Acanthodactylus boskianus*, (B) *Acanthodactylus hardyi*, (C) *Acanthodactylus opheodurus*, (D) *Acanthodactylus schmidtii*, (E) *Mesalina brevirostris*, (F) *Mesalina* lineage 4, (G) *Mesalina saudiarabica*, (H) *Diplometopon zarudnyi*.

trends in the reserve. In addition, this survey provides a benchmark for comparisons to other reserves in the Arabian Peninsula.

The KARNR lies in an area predicted to have high reptile species richness of between 20 to 40 species (Alatawi et al. 2020; Šmíd et al. 2021). The 34 species

confirmed to occur in the reserve are within these bounds of the richness projection, but more species may potentially occur here, particularly in the southern areas (Alatawi et al. 2020; Šmíd et al. 2021). Species richness was not evenly distributed over the different broad habitat types. Hills, outcrops, and escarpments had the highest

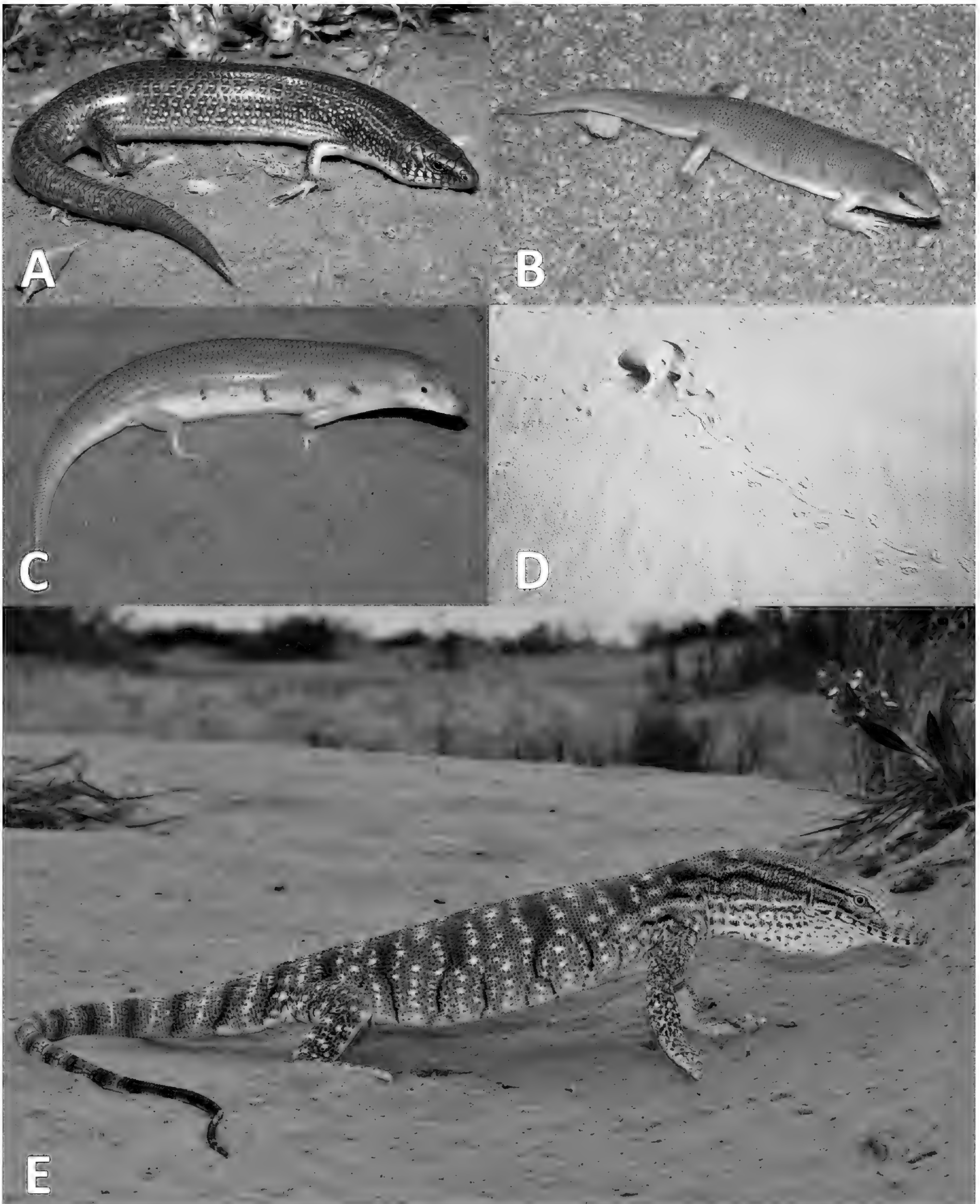


Fig. 8. (A) *Chalcides ocellatus*, (B) *Scincus mitranus*, (C) *Scincus conirostris*, (D) *Scincus conirostris* tracks, (E) *Varanus griseus*.

diversity in terms of species richness, followed by Wadis and rawdhats, and Plains. Dunes and dune valleys had the lowest species richness. This trend can be attributed to the habitat heterogeneity hypothesis, which states that regions with higher habitat heterogeneity have higher species diversity (MacArthur and MacArthur 1961).

Additional surveys within the reserve are likely to yield new species, but several of the species encountered during the surveys are also undergoing taxonomic revision. For example, tentative molecular analyses indicate that more than one species of the *Ptyodactylus hasselquistii* complex may occur within the KARNR,

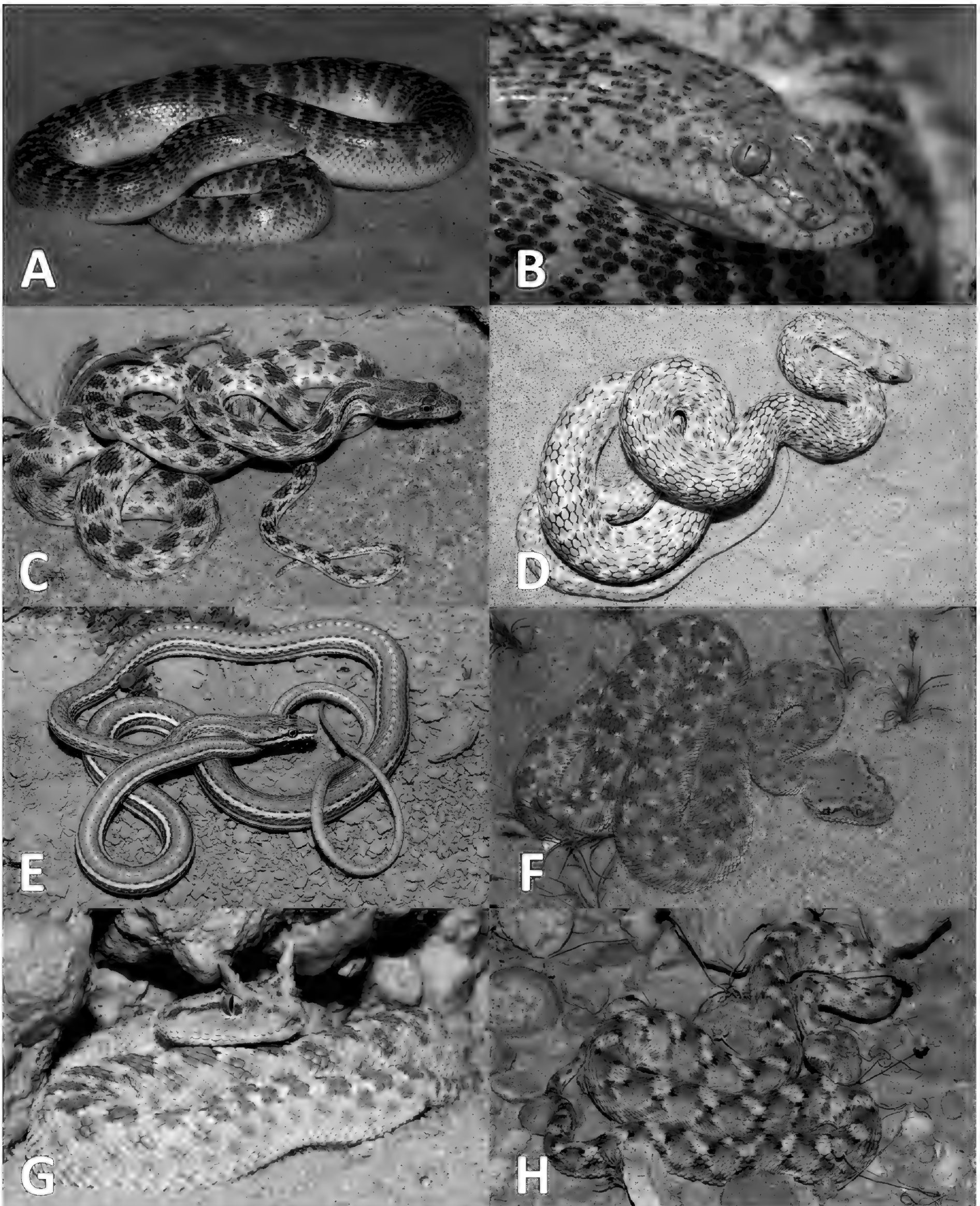


Fig. 9. (A) *Eryx jayakari*, (B) *Eryx jayakari*, (C) *Spalerosophis diadema*, (D) *Malpolon moilensis*, (E) *Psammophis schokari*, (F) *Cerastes gasperettii*, (G) *Cerastes gasperettii*, (H) *Echis coloratus*.

thus the reserve's species list will need to be updated as these new taxa are formally described. Likewise, the *Mesalina guttulata* species complex (recorded during our survey as *Mesalina* lineage 4 following Sindaco et al. 2018) is also undergoing taxonomic revision.

The four species not previously recorded from the

KARNR region are *Trapelus agnetae*, *Hemidactylus granosus*, *Mesalina saudiarabica*, and *Diplometopon zarudnyi*. The occurrence of *M. saudiarabica* was confirmed by DNA barcoding, with three samples of this species occurring in the Ad Dahna sand stretch of the reserve. The occurrence of this species was unexpected

Reptiles of the King Abdulaziz Royal Nature Reserve

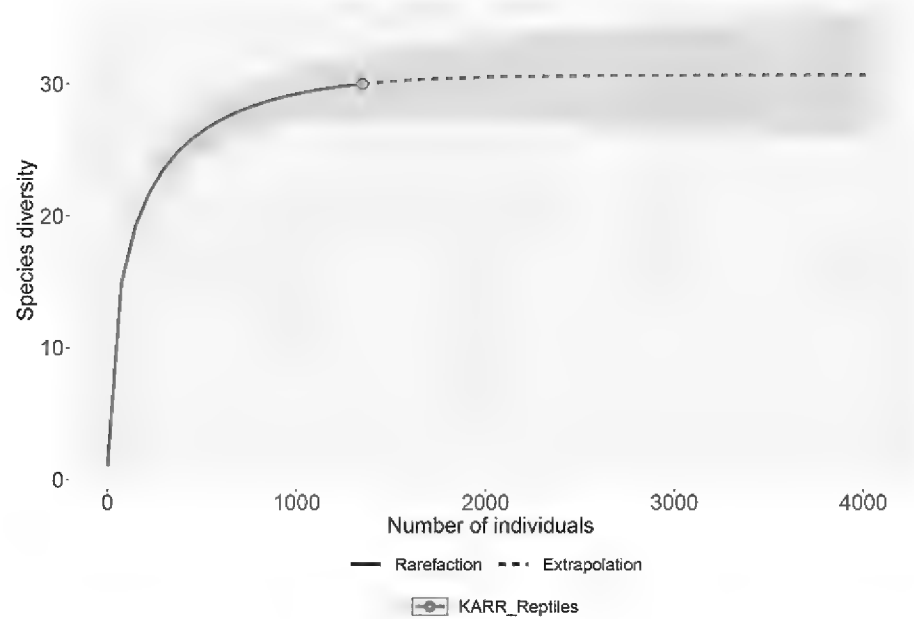


Fig. 10. Predictive species accumulation curve generated with the R package iNEXT. The solid line represents observations made during the 2022/23 survey period and the stippled line represents the extrapolation of the data to double the survey effort.

based on its previously known distribution that was restricted to the western deserts of Saudi Arabia (Šmíd et al. 2017). Not only do these records represent a significant range extension of more than 600 km to the north-east from the previously known range, but they also indicate the need for more detailed taxonomic investigations and herpetofaunal surveys in this understudied region as well as in other parts of central Saudi Arabia that remain largely under-surveyed.

One of the most frequently detected species within the KARNR is *Uromastyx aegyptia*. This species is widespread throughout the reserve and occurs in all broad habitat types. *Uromastyx aegyptia* is listed as Vulnerable by the IUCN, with threats relating to

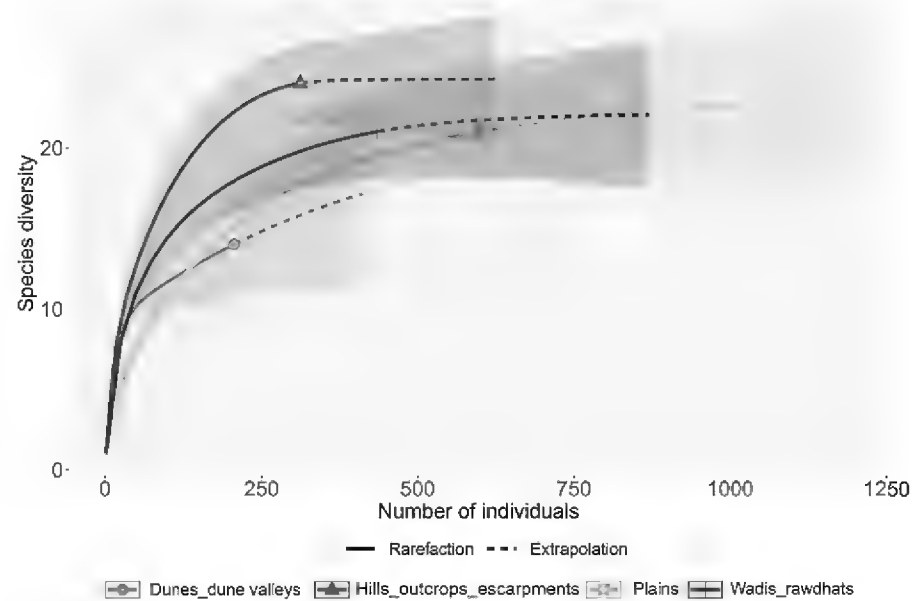


Fig. 11. Predictive species accumulation curves generated with the R package iNEXT for four different habitat types (Dunes and dune valleys; Hills, outcrops, and escarpments; Plains; and Wadis, rawdhats, and pans). The solid lines represent observations made during the surveys and the stippled lines represent the extrapolation of the data to double the survey effort.

increasing anthropogenic pressures such as agricultural expansion, overgrazing, and poaching (IUCN 2023). Since the reserve was established, proactive measures have been taken to prevent habitat degradation, and hunting of *U. aegyptia* within the reserve borders has been made illegal. This highlights the role of KARNR as an important conservation area for reptiles in general, but especially for this Threatened species. Climate change is thought to be an additional threat to *U. aegyptia*, with ecological niche modelling projections indicating that habitat suitability across the Arabian Peninsula will decrease by 2070 (Kechnebbou et al. 2021). Under the worst-case scenario, only extremely limited areas within the reserve are predicted to remain suitable for *U.*

Table 2. Summary of species diversity calculations performed using the species accumulation curves generated with the R package iNEXT. For the KARNR as a whole and each of the broadscale habitat types, the diversity indices (Species richness, Shannon, and Simpson) are summarized in terms observed and estimated diversity with the standard error and upper and lower confidence levels. The iNEXT completeness percentage was calculated based on the observed versus estimated diversity.

Assemblage	Diversity	Observed	Estimator	Standard error	LCL	UCL	iNEXT completeness
KARNR	Species richness	31.00	31.67	3.15	31.00	37.84	97.88
	Shannon diversity	8.39	8.48	0.29	7.92	9.04	98.94
	Simpson diversity	4.75	4.76	0.17	4.43	5.09	99.79
Dunes and dune valleys	Species richness	14.00	21.96	6.29	14.00	34.29	63.75
	Shannon diversity	8.17	8.60	0.59	7.45	9.75	94.99
	Simpson diversity	6.30	6.47	0.54	5.40	7.53	97.42
Hills, outcrops, and escarpments	Species richness	24.00	24.25	5.97	24.00	35.95	98.97
	Shannon diversity	9.68	10.07	0.65	8.80	11.34	96.11
	Simpson diversity	6.33	6.44	0.50	5.46	7.42	98.29
Plains	Species richness	21.00	23.66	7.34	21.00	38.05	88.75
	Shannon diversity	4.89	4.99	0.31	4.39	5.60	97.94
	Simpson diversity	3.39	3.40	0.14	3.13	3.68	99.60
Wadis, rawdhats, and pans	Species richness	21.00	22.12	4.35	21.00	30.65	94.93
	Shannon diversity	6.37	6.54	0.44	5.69	7.40	97.41
	Simpson diversity	3.37	3.39	0.25	2.90	3.88	99.45

aegyptia, while suitability remains across KARNR for the best-case scenario (Kechnebbou et al. 2021). As such, the KARNR is an important sanctuary for the long-term protection of this species. However, future conservation plans for the reserve should focus not only on mitigating the impacts of anthropogenic pressures, but also on developing a monitoring program to identify potential range reductions within the reserve borders in the context of climate change.

The other reptile of conservation concern found within the reserve is *Tropicolotes wolfgangboehmei*, which is listed as Data Deficient by the IUCN and considered one of the world's most range-restricted reptiles (IUCN 2023; Meiri et al. 2018). This species was previously known from only three localities (Šmíd et al. 2021). We observed two individuals of this species during our survey, one at a previously known site within the 10 km buffer (300 m from the reserve boundary), while the other represents a new locality record (25 km north-east from the known site). Both records are from the Hills, outcrops, and escarpments habitat type. Future surveys in the KARNR should target this range-restricted species at additional sites and investigate its ecological requirements to assess the extent of its protection within the reserve.

In conclusion, our herpetological survey in the KARNR contributes significantly to our knowledge of the reptile diversity in east-central Saudi Arabia and provides a robust baseline for the region. The occurrences of 34 reptile species have been confirmed for the reserve, including four not previously recorded. The survey effort assessment, through predictive species accumulation curves and diversity indices, indicates that our methodology was adequate by documenting an estimated 97.89% of reptile species present in the KARNR. However, we acknowledge that further field and genetic investigations may reveal additional species.

The distribution of species richness across different broad habitat types reveals insights into the importance of habitat heterogeneity in supporting reptile diversity. The KARNR is a protected area of significant conservation importance, both at the regional and global scales. Its protection is beneficial for species of conservation concern such as *Uromastix aegyptia* (VU) and *Tropicolotes wolfgangboehmei* (DD), and for the more common and widespread reptile species and assemblages in the region. Ongoing taxonomic revisions and the potential impacts of climate change on sensitive species emphasize the need for more rigorous monitoring and for the implementation of adaptive conservation strategies. Our findings not only contribute significantly to the herpetofaunal knowledge of the Arabian Peninsula but also serve as a foundational resource for conservation planning and assessments, long-term monitoring, and regional comparative studies.

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Literature Cited

- Alatawi AS, Gilbert F, Reader T. 2020. Modelling terrestrial reptile species richness, distributions, and habitat suitability in Saudi Arabia. *Journal of Arid Environments* 178: 104153.
- Almalki KA, Al Mosallam MS, Aldaajani TZ, Al-Namazi AA. 2022. Landform characterization of Saudi Arabia: towards a geomorphological map. *International Journal of Applied Earth Observation and Geoinformation* 112: 102945.
- Aloufi AA, Amr ZS. 2015. On the herpetofauna of the Province of Tabuk, northwest Saudi Arabia. *Herpetozoa* 27(3/4): 147–158.
- Aloufi AA, Amr ZS, Abu Baker MA. 2021. Reptiles and amphibians of Al Madinah Al Munawwarah Province, Saudi Arabia. *Russian Journal of Herpetology* 28(3): 123–137.
- Aloufi AA, Amr ZS, Baker MA. 2022. Reptiles from ‘Uruq Bani Ma’arid and Harat al Harrah protected areas in Saudi Arabia: reptiles from two protected areas in Saudi Arabia. *Herpetology Notes* 15: 483–491.
- Aloufi AA, Amr ZS, Abu Baker MA, Hamidan N. 2019. Diversity and conservation of terrestrial, freshwater, and marine reptiles and amphibians in Saudi Arabia. *Amphibian & Reptile Conservation* 13(2) [General Section]: 181–202 (e204).
- Al-Sadoon MK. 1988. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. II. The lizard and amphisbaenian fauna of Riyadh Province. *Bulletin of the Maryland Herpetological Society* 24(3): 58–76.
- Al-Sadoon MK, Paray BA, Al-Otaibi HS. 2016. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. V. The lizard fauna of Turaif region. *Saudi Journal of Biological Sciences* 23(5): 642–648.
- Al-Sadoon MK. 1989. Survey of the reptilian fauna of

- the Kingdom of Saudi Arabia. I. The snake fauna of the central region. *Journal of King Saud University - Science* 1(1): 2.
- Al-Sadoon MK, Paray BA, Al-Otaibi H. 2017. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. VI. The snake fauna of Turaif region. *Saudi Journal of Biological Sciences* 24(4): 925–928.
- Alshammari AM, Busais SM, Ibrahim AA. 2017. Snakes in the Province of Ha'il, Kingdom of Saudi Arabia, including two new records. *Herpetozoa* 30(1–2): 59–63.
- Alshammari AM, Busais SM. 2020. Distribution of snakes in Ha'il Province, Saudi Arabia, with an identification key to the species. *Russian Journal of Herpetology* 27(1): 5–10.
- Alshammari AM, Ibrahim AA. 2015. Lizards and snakes in the historical Faid protected area (Faïd Hema), Ha'il region, Saudi Arabia. *Herpetological Conservation and Biology* 10(3): 1,021–1,029.
- Carranza S, Els J, Burriel-Carranza B. 2021. *A Field Guide to the Reptiles of Oman*. Bloomsbury Publishing, New York, New York, USA. 226 p.
- Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, Ellison AM. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* 84(1): 45–67.
- Chao A, Jost L. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 1(12): 2,533–2,547.
- Cox NA, Mallon D, Bowles P, Els J, Tognelli MF. 2012. *The Conservation Status and Distribution of Reptiles of the Arabian Peninsula*. IUCN, Cambridge, United Kingdom and Gland, Switzerland, and Environment and Protected Areas Authority, Sharjah, United Arab Emirates. 49 p.
- Cressey ER, Measey GJ, Tolley KA. 2015. Fading out of view: the enigmatic decline of Rose's Mountain Toad *Capensibufo rosei*. *Oryx* 49(3): 521–528.
- Cunningham PL. 2010. Checklist of terrestrial reptiles in three protected areas in the Kingdom of Saudi Arabia. *Herpetological Review* 41(1): 25–28.
- Farag AA, Banaja AA. 1980. Amphibians and reptiles from the western region of Saudi Arabia. *Bulletin of Science, King Abdulaziz University* 4: 5–29.
- Ficetola GF, Bonardi A, Sindaco R, Padoa-Schioppa E. 2013. Estimating patterns of reptile biodiversity in remote regions. *Journal of Biogeography* 40(6): 1,202–1,211.
- Gasperetti J. 1988. Snakes of Arabia. *Fauna of Saudi Arabia* 9: 169–450.
- Ghazanfar SA, Fisher M, Editors. 1998. *Vegetation of the Arabian Peninsula (Number 25)*. Springer Science & Business Media, Berlin, Germany. 373 p.
- Hsieh TC, Ma KH, Chao A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7(12): 1,451–1,456.
- International Union of Conservation for Nature (IUCN). 2023. IUCN Red List of Threatened Species. IUCN: Gland, Switzerland. Available: <https://www.iucnredlist.org/> [Accessed: 7 July 2023].
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1,647–1,649.
- Kechnebbou M, de Carvalho DL, da Silva PH, Silva DP. 2021. Global warming drives range shifts in spiny-tailed lizards (Squamata: Agamidae: *Uromastix*) in the African and Arabian deserts. *Journal of Arid Environments* 191: 104522.
- Kordges T. 1998. Die reptilian Fauna des Thumama Nature Park bei Riyadh, Saudi Arabien. *Faunistische Abhandlungen Staatliches Museum für Tierkunde Dresden* 21: 67–83.
- MacArthur RH, MacArthur JW. 1961. On bird species diversity. *Ecology* 42(3): 594–598.
- Margules CR, Pressey RL. 2000. Systematic conservation planning. *Nature* 405(6783): 243–253.
- Maritz B, Penner J, Martins M, Crnobrnja-Isailovic J, Spear S, Alencar LRV, Sigala-Rodriguez J, Messenger K, Clark RW, Soorae P, et al. 2016. Identifying global priorities for the conservation of vipers. *Biological Conservation* 204: 94–102.
- Meiri S, Bauer AM, Allison A, Castro-Herrera F, Chirio L, Colli G, Roll U. 2018. Extinct, obscure, or imaginary: the lizard species with the smallest ranges. *Diversity and Distributions* 24(2): 262–273.
- Mendes DM, de Freitas Leão R, Toledo LF. 2015. Drift fences in traps: theoretical evidence of effectiveness of the two most common arrays applied to terrestrial tetrapods. *Natureza & Conservação* 13(1): 60–66.
- Mihoub JB, Henle K, Titeux N, Brotons L, Brummitt NA, Schmeller DS. 2017. Setting temporal baselines for biodiversity: the limits of available monitoring data for capturing the full impact of anthropogenic pressures. *Scientific Reports* 7(1): 41591.
- Moreno CE, Halffter G. 2001. On the measure of sampling effort used in species accumulation curves. *Journal of Applied Ecology* 38: 487–490.
- Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* 11(5): 1,633–1,644.
- QGIS Development Team. 2023. QGIS Geographic Information System. Open-Source Geospatial Foundation Project. Available: <http://qgis.osgeo.org> [Accessed: 17 March 2022].
- R Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <https://www.R-project.org/> [Accessed: 10 February 2022].

- Salvador A. 1982. *A Revision of the Lizards of the Genus Acanthodactylus* (Sauria: Lacertidae). *Bonner Zoologische Monographien, Nummer 16*. Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany. 167 p.
- Sindaco R, Simo-Riudalbas M, Sacchi R, Carranza S. 2018. Systematics of the *Mesalina guttulata* species complex (Squamata: Lacertidae) from Arabia with the description of two new species. *Zootaxa* 4429(3): 513–547.
- Sinervo B, Mendez-De-La-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagrán-Santa Cruz M, Lara-Resendiz R, Martínez-Méndez N, Calderón-Espinosa ML, Meza-Lázaro RN, et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328(5980): 894–899.
- Šmíd J, Moravec J, Gvoždík V, Štundl J, Frynta D, Lymberakis P, Kapli P, Wilms T, Schmitz A, Shobrak M, et al. 2017. Cutting the Gordian knot: phylogenetic and ecological diversification of the *Mesalina brevirostris* species complex (Squamata, Lacertidae). *Zoologica Scripta* 46(6): 649–664.
- Šmíd J, Sindaco R, Shobrak M, Busais S, Tamar K, Aghová T, Simó-Riudalbas M, Tarroso P, Geniez P, Crochet PA, et al. 2021. Diversity patterns and evolutionary history of Arabian squamates. *Journal of Biogeography* 48(5): 1,183–1,199.
- Soga M, Gaston KJ. 2018. Shifting baseline syndrome: causes, consequences, and implications. *Frontiers in Ecology and the Environment* 16(4): 222–230.
- Tamar K, Uvizl M, Shobrak M, Almutairi M, Busais S, Salim AFA, AlGethami RHM, AlGethami AR, Alanazi ASK, Alsubaie SD, et al. 2023. A new species of *Pseudotrapelus* (Reptilia: Squamata: Agamidae) from Central Arabia. *Vertebrate Zoology* 73: 1,033–1,045.
- Thompson GG, Thompson SA, Withers PC, Fraser J. 2007. Determining adequate trapping effort and species richness using species accumulation curves for environmental impact assessments. *Austral Ecology* 32(5): 570–580.
- Van Rooyen M, Van Rooyen N, Gaugris JY. 2023. *Study of the Ecological Aspects – Botanical Studies of the Master Plan Section of the King Abdulaziz Royal Nature Reserve*. FLORA FAUNA & MAN, Ecological Services Limited, Tortola, British Virgin Islands. 121 p.
- Vincent P. 2008. *Saudi Arabia: an Environmental Overview*. CRC Press, London, United Kingdom. 332 p.
- Zipkin EF, DiRenzo GV, Ray JM, Rossman S, Lips KR. 2020. Tropical snake diversity collapses after widespread amphibian loss. *Science* 367(6479): 814–816.



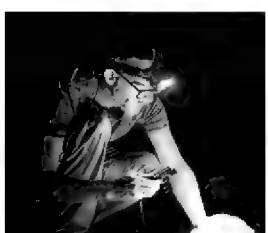
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Reptiles of the King Abdulaziz Royal Nature Reserve



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Abdullah M. Alowaifeer is an environmental scientist interested in conservation, environmental contaminants, environmental risk assessment, and environmental rehabilitation. While pursuing his Ph.D. in Ecology at Montana State University (Bozeman, Montana, USA), he was involved in various projects that led him to gain theoretical and technical expertise in experimental design, sampling, and sample analysis. Abdullah did his Ph.D. thesis project on elemental cycling in Yellowstone National Park in the USA. His work was published and recognized as one of the top 10 Exceptional Papers of 2023 in the journal *Environmental Toxicology and Chemistry*. Abdullah has published over 20 scientific papers on various topics related to his work interests. He is currently the General Manager of Environmental Sustainability at King Abdulaziz Royal Nature Reserve in Saudi Arabia.



Jerome Gaugris is an accredited conservation scientist, ecologist, and wildlife manager. He is committed to providing practical, financially viable, and socially acceptable solutions that integrate human activities compatible with the long-term goal of biodiversity and ecosystem services conservation. Jerome has over 20 years of international professional experience focused on designing and implementing more than 100 scientifically grounded studies for ecological investigations and the sustainable use of renewable natural resources. Jerome is a vetted expert on Biodiversity and Sustainable Land Management for the United Nations Development Programme (UNDP) Africa, Caribbean and Pacific (ACP), and a vetted expert on Reduced Emissions from Deforestation and Forest Degradation (REDD+) for the Central African Forestry Initiative (CAFI).



Introductory page. *Pantherophis bairdi* (Yarrow, 1880). The distribution of Baird's Ratsnake extends from “the Edwards Plateau and Big Bend region in southwestern Texas southward through central Coahuila and Nuevo León to the Sierra Madre Oriental of southern Tamaulipas” (Heimes, 2016: 122); this species also occurs in southeastern Chihuahua. This individual was photographed in scrub vegetation at Sierra Rica, a Natural Protected Area in the municipality of Manuel Benavides, Chihuahua. Wilson et al. (2013a) determined its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN has considered its conservation status as Least Concern, but this species is not listed by SEMARNAT. Photo by Sara G. Sáenz-González.



The herpetofauna of Chihuahua, Mexico: composition, distribution, and conservation status

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Abstract.—The herpetofauna of the Mexican state of Chihuahua presently consists of 186 species, including 35 anurans, five salamanders, 133 squamates, and 13 turtles. The members of the herpetofauna are distributed among nine physiographic regions, ranging from 58 species in the Sierras y Llanuras de Durango to 128 in the Gran Meseta y Cañones Chihuahuenses. The number of species shared between the areas ranges from 14 to 70. The Coefficient of Biogeographic Resemblance values range from 0.23 to 0.83. A UPGMA dendrogram indicates that the closest relationships among the nine physiographic regions are those adjacent to one another, in a series of swaths of two to three regions generally oriented in a northwestern to southeastern direction from the southwestern to the northeastern sectors of the state. The level of endemism in Chihuahua is relatively limited and consists of 61 country endemics and one state endemic species. The distributional categorization of the entire herpetofauna consists of 61 country endemics, one state endemic, 121 non-endemics, and three non-native species. We placed the 121 non-endemic species in the following distributional categories: MXUS (108), USCA (six), MXCA (three), MXSA (three), and USSA (one). The principal environmental threats to the herpetofauna of Chihuahua are land conversion and habitat loss, water management (quality and quantity), invasive species, climate change, fires, illegal trade, diseases and parasites, on and off-road activities, mining, pollution, human consumption, animal grazing and agriculture, fear and confusion by people, and miscellaneous threats. We evaluated the conservation status of each native species by using the SEMARNAT, IUCN, and EVS systems, of which the EVS proved to be the most useful. We used the Relative Herpetofaunal Priority method to rank the physiographic regions and found that the Gran Meseta y Cañones Chihuahuenses is of the greatest importance. Finally, we provide a set of conclusions and recommendations to help improve the future protection of the herpetofauna of Chihuahua.

Keywords. Anurans, caudates, conservation status, physiographic regions, protected areas, protection recommendations, squamates, turtles

Resumen.—La herpetofauna del estado mexicano de Chihuahua actualmente consiste de 186 especies, que incluye 35 anuros, cinco salamandras, 133 escamados y 13 tortugas. Los miembros de la herpetofauna se distribuyen en nueve regiones fisiográficas, desde 58 especies en las Sierras y Llanuras de Durango hasta 128 en la Gran Meseta y Cañones Chihuahuenses. El número de especies compartidas entre las áreas oscila entre 14 y 70. Los valores del Coeficiente de Semejanza Biogeográfica oscilan entre 0,23 y 0,83. Un dendrograma de la UPGMA indica que las relaciones más cercanas entre las nueve regiones fisiográficas son aquellas que son adyacentes, en una serie de franjas de dos a tres regiones generalmente orientadas en dirección noroeste a sureste, desde el sector suroeste al noreste del estado. El nivel de endemismo en Chihuahua es relativamente limitado y consiste de 61 especies endémicas a nivel nacional y una especie endémica a nivel estado. La categorización distribucional de toda la herpetofauna consiste de 61 especies endémicas en el país, una endémica al estado, 121 no endémicas y tres especies no nativas. Colocamos las 121 especies no endémicas en las siguientes categorías de distribución: MXUS (108), USCA (seis), MXCA (tres), MXSA (tres) y USSA (una). Las principales amenazas ambientales a la herpetofauna de chihuahua son: Conversión de suelo y pérdida de hábitat; gestión del agua (calidad y cantidad); especies invasoras; cambio climático; incendios; comercio ilegal; enfermedades y parásitos; actividades dentro y fuera de la carretera; minería; contaminación; consumo humano; pastoreo de animales y agricultura; miedo y confusión por parte de la gente; y amenazas diversas. Evaluamos el estado de conservación de cada especie nativa utilizando los sistemas de SEMARNAT, UICN y EVS, de los cuales el EVS resultó ser el más útil. Utilizamos el método de Prioridad Relativa Herpetofaunística para determinar la región fisiográfica de mayor importancia, es decir, la Gran Meseta y Cañones Chihuahuenses. Finalmente, brindamos un conjunto de conclusiones y

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recomendaciones para ayudar a mejorar la protección futura de la herpetofauna de Chihuahua.

Palabras Claves. Anuros, áreas protegidas, caudados, escamosos, estatus de conservación, recomendaciones de protección, regiones fisiográficas, tortugas

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“I’ve been buried in life. My own, of course, but much of my life’s work has focused on the lives of animals, plants, and microbes and their evolution and behavior. It has included the evolution and behavior that shapes the individual lives of my favorite mammal, Homo sapiens. My life also has entailed efforts to influence society to move rapidly in the direction of improving all life, and lives, by limiting human population growth, increasing racial, gender, and economic equity, and conserving our environmental life-support systems abundantly supplied with nonhuman lives. A vision for a better and more sustainable life for all people living on the one-and-only habitable planet in our solar system has arisen naturally from my lifelong studies of butterflies and my distress at the loss of their natural habitats.”

Preface in *Life: A Journey through Science and Politics* by Paul R. Ehrlich (2023)

Introduction

Chihuahua is the largest of the 32 federal entities in Mexico (31 states + Mexico City), and with an area of 247,412.6 km², which represents 12.6% of the country’s surface. Sonora, Chihuahua’s neighbor to the west, is the second largest state in the country, with a surface area of 179,354.7 km² (9.1% of the country’s surface) and Coahuila, its neighbor to the east, is the third largest with an area of 151,594.8 km², representing 7.7% of the country’s surface (INEGI 2021a). Chihuahua is one of six Mexican states that borders the United States of America (USA), as it is bordered by portions of New Mexico and Texas (Rand McNally 1998).

To the north, the state of Chihuahua is bordered by the USA, to the east by Coahuila de Zaragoza, to the south by Durango, to the southwest by Sinaloa, and to the west by Sonora. In 2020, the total population of Chihuahua was 3,741,869, which represented 3.0% of the national population (INEGI 2022).

The states with the lowest density of inhabitants/km² in Mexico are Chihuahua, Durango, and Baja California Sur. Chihuahua has a density of 15.1 inhabitants/km², and ranks 30th, after Durango (14.9 inhabitants/km²) and Baja California Sur (10.8 inhabitants/km²). The low human population density of Chihuahua likely is due to the location of the Chihuahuan Desert and the highlands of the Sierra Madre Occidental (see Addendum).

According to INEGI (2021b), Chihuahua includes four terrestrial ecoregions of Mexico: North American deserts (45.8%), temperate sierras (28.2%), southern semi-arid elevations (23.4%), and hot dry tropical forest (2.6%). The biophysical characteristics in the state of Chihuahua provide adequate conditions for biodiversity at the ecosystem, species, and genetic levels (Reyes-Gomez and Valero-Padilla 2014). The development and maintenance of a biodiversity information system for Chihuahua is conducted by the organizations that manage scientific knowledge and information (CONABIO and SEDUE, Gobierno de Chihuahua 2015).

The objectives of this paper are to update the list of amphibians and reptiles of Chihuahua, to discuss their

distribution by physiographic subprovince, to identify the environmental threats impinging on them, and to document their conservation status, as we have done for the other entries in the Mexican Conservation Series, as discussed below.

Materials and Methods

Our Taxonomic Position

In this paper, we follow the same taxonomic position as explained in previous works on other portions of Mesoamerica (Johnson et al. 2015a,b; Mata-Silva et al. 2015; Terán-Juárez et al. 2016; Woolrich-Piña et al. 2016; Nevárez-de los Reyes et al. 2016; Cruz-Sáenz et al. 2017; Gonzalez-Sánchez et al. 2017; Woolrich-Piña et al. 2017; Lazcano et al. 2019; Ramírez-Bautista et al. 2020; Torres-Hernández et al. 2021; Cruz Elizalde et al. 2022; Barragán-Vázquez et al., 2022; Leyte-Manrique et al., 2022; Peralta-García et al., 2023). Johnson et al. (2015a) can be consulted for a statement of this position, with special reference to the subspecies concept. In addition, we adopted the recent change in the family name for the anoline lizards promulgated by De Queiroz (2022, 2023), i.e., from Dactyloidae to Anolidae.

System for Determining Distributional Status

We used the system developed by Alvarado-Díaz et al. (2013) for the herpetofauna of Michoacán to ascertain the distributional status of members of the herpetofauna of Chihuahua. Subsequently, Mata-Silva et al. (2015), Johnson et al. (2015a), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016), Nevárez-de los Reyes et al. (2016), Cruz-Sánchez et al. (2017), González-Sánchez et al. (2017), Woolrich-Piña et al. (2017), Lazcano et al. (2019), Ramírez-Bautista et al. (2020), Torres-Hernández et al. (2021), Cruz Elizalde et al. (2022), Barragán-Vázquez et al. (2022), Leyte-Manrique et al. (2022), and Peralta-García et al. (2023), also used this system, which consists of the following four categories: SE = endemic to Chihuahua; CE = endemic to Mexico; NE = not endemic to Mexico; and NN = non-native in Mexico.

The herpetofauna of Chihuahua, Mexico

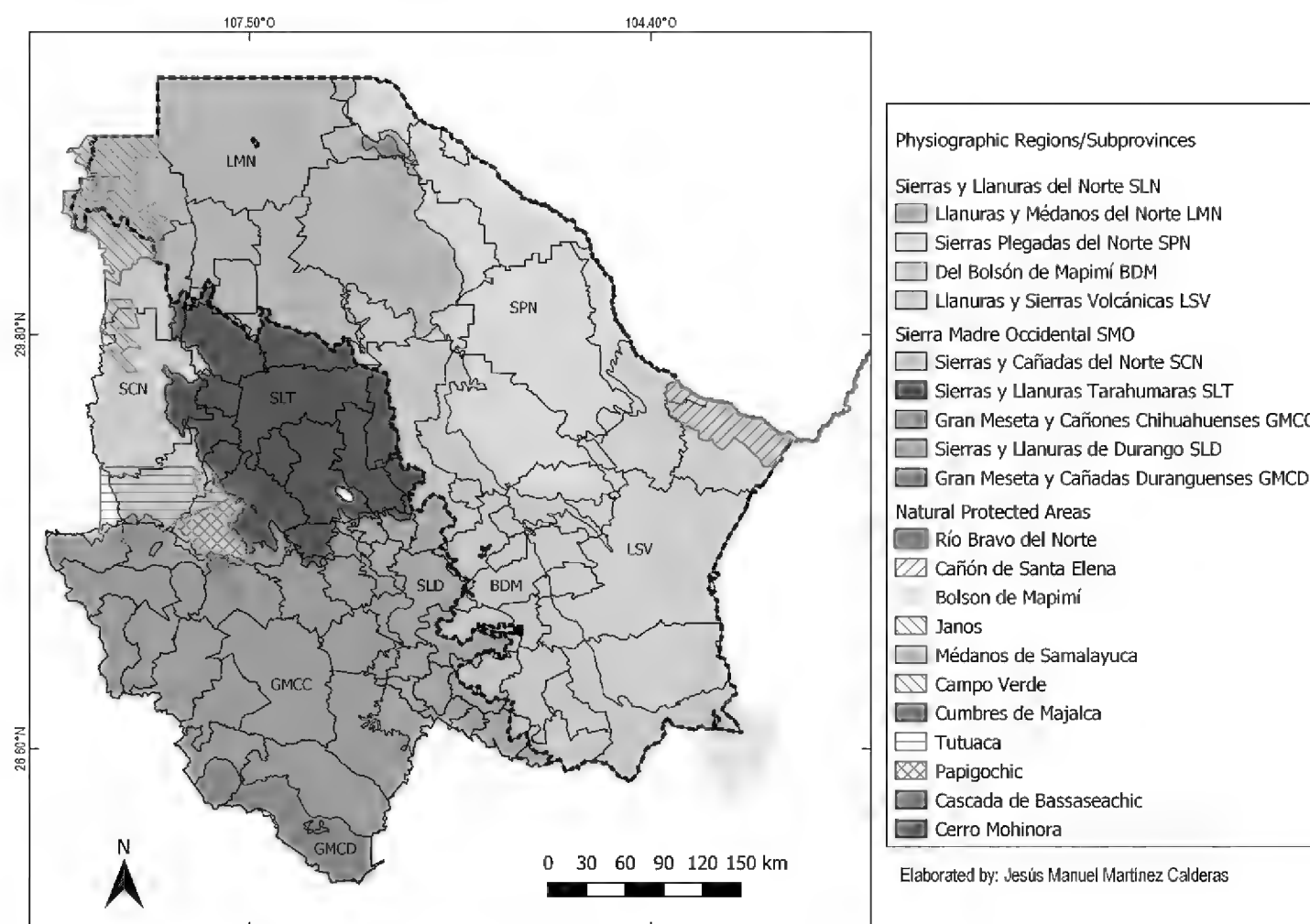


Fig. 1. Physiographic regions and Natural Protected Areas (NPAs) in the state of Chihuahua, Mexico. The northwest to southeast oriented solid black line separates the subprovinces of the Sierra Madre Oriental from those of the Sierras y Llanuras del Norte.

Systems for Determining Conservation Status

To evaluate the conservation status of the herpetofauna of Chihuahua, we employed the three systems (i.e., SEMARNAT, IUCN, and EVS) used by Alvarado-Díaz et al. (2013), Mata-Silva et al. (2015), Johnson et al. (2015a), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016), Nevárez-de los Reyes et al. (2016), Cruz-Sánchez et al. (2017), González-Sánchez et al. (2017), Woolrich-Piña et al. (2017), Lazcano et al. (2019), Ramírez-Bautista et al. (2020), Torres-Hernández et al. (2021), Cruz Elizalde et al. (2022), Barragán-Vázquez et al. (2022), Leyte-Manrique et al. (2022), and Peralta-García et al. (2023). Detailed descriptions of these three systems appear in earlier papers in this series and are not repeated here.

The Mexican Conservation Series

The Mexican Conservation Series (MCS) began in 2013 with a study of the herpetofauna of Michoacán (Alvarado-Díaz et al. 2013), as part of a set of five papers designated as the “Special Mexico Issue” published in *Amphibian & Reptile Conservation*. The basic format of the entries in the MCS was established in that paper, i.e., to examine the composition, physiographic distribution, and conservation status of the herpetofauna of a given Mexican state or group of states. Two years later, the MCS resumed with a paper on the herpetofauna of Oaxaca (Mata-Silva et al. 2015), and that year Johnson et al. (2015a) authored a paper on the herpetofauna of Chiapas. Three entries in the MCS appeared the following year, on Tamaulipas (Terán-Juárez et al. 2016), Nayarit (Woolrich-Piña et al. 2016), and Nuevo León (Nevárez-de los Reyes et al. 2016). Three more entries, on Jalisco (Cruz-Sáenz et al. 2017), the Mexican Yucatan Peninsula

(González-Sánchez et al. 2017), and Puebla (Woolrich-Piña et al. 2017) were published in 2017. These entries were followed by one on Coahuila (Lazcano et al. 2019) and another on Hidalgo (Ramírez-Bautista et al. 2020). In the ensuing two years, papers on Veracruz (Torres-Hernández et al. 2021), Querétaro (Cruz-Elizalde et al. 2022), Tabasco (Barragán-Vázquez et al. 2022), Guanajuato (Leyte-Manrique et al. 2022), and finally Baja California Peninsula (García-Peralta et al. 2023) were published. Thus, this paper on the herpetofauna of Chihuahua is the 17th (and final) entry in this series.

Physiography and Climate

Physiographic Regions

Two of the 15 physiographic provinces in Mexico are present in Chihuahua (INEGI 2021b), the Sierra Madre Occidental (SMO), with five subprovinces, and the Sierra y Llanuras del Norte (SLN), with four subprovinces (Fig. 1). Below we describe the physiography of the provinces and subprovinces for Chihuahua, according to INEGI (1999, 2003), as well as their vegetation.

Sierra Madre Occidental (SMO) Province and its five subprovinces

This province (Fig. 1) begins with only a tiny portion on the border with the USA, and it extends southeastward to end in the vicinity of the “Eje Neovolcánico” province, located south of the state of Chihuahua. The SMO is the longest mountain system in Mexico, with average elevations ranging from 2,000 to 2,500 m (Rzedowski 2006), although in Chihuahua they reach up to 3,300 m. This province covers 43.4% of the state’s surface area and includes five subprovinces: Sierras y Cañadas del



Fig. 2. Sierras y Cañadas del Norte (SCN). Near the entrance of Cueva de la Olla, showing Parry's Agave (*Agave parryi*) and pine trees, Casas Grandes, Chihuahua. Photo by Ana Gatica-Colima.



Fig. 3. Sierras y Llanuras Tarahumaras (SLT). Cumbres de Majalca National Park, “Cañón de la Gringa,” Chihuahua. Note the volcanic and volcanoclastic rocks within the pine-oak vegetation. Photo by Ramón I. Miramontes-Cinco.

Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Sierras y Llanuras de Durango (SLD), Gran Meseta y Cañones Chihuahuenses (GMCC), and Gran Meseta y Cañadas Duranguenses (GMCD). This region includes dry and semi-dry to temperate and semi-cold climates in the highest areas (INEGI 2003).

Sierras y Cañadas del Norte (SCN). According to INEGI (1999), the dominant topoform in this subprovince consists of high elevation sierras such as the Sierra San Luis (900–2,500 m). Some of the municipalities in this subprovince are Madera, Casas Grandes, Guerrero, and Temosachi. This region consists of a sierra with steep slopes, which physiographically is classified as a high sierra with ravines (Fig. 2). To the west it reaches elevations of 1,000 m, but to the east the elevations are higher than 2,000 m, with a maximum of 2,700 m. The terrain is characterized by acidic volcanic rock with large basaltic layers present on the San Luis, Hachita Hueca, and La Breña mountains, among others. Located in the northwest of the SMO province, it occupies 7.3% of the state's surface. This subprovince is drained by several

streams, including the Sirupa, Tutuaca, and Rio Chico, which are tributaries of the Aros River; the Chuhuichupa, a tributary of the Bavispe River; and the San Pedro and Piedras Verdes rivers (INEGI 2003).

The principal vegetation in the communities of this subprovince consists of *Pinus engelmannii*, *P. durangensis*, *P. arizonica*, *Abies religiosa*, *Pseudotsuga menziessi*, *Quercus sideroxyla*, *Q. arizonica*, and *Q. rugosa*. To a lesser degree, elements of chaparral, grassland, and low deciduous forest, as well as agricultural areas, occur in this region (Alva-Álvarez et al. 2018).

Sierras y Llanuras Tarahumaras (SLT). Located in the west-central portion of Chihuahua (Fig. 3), this subprovince is characterized by high and low elevation topoforms that are structurally oriented in a northwest to southeast direction. The subprovince is highly modified in its relief, as hills that are associated with ravines (“cañadas”) and slopes (“bajadas”) have formed in lower topographic areas where valleys and plains are the dominant topoforms, such as in the extensive



Fig. 4. Sierras y Llanuras de Durango (SLD). Rancho La Catorce, Santa Bárbara, Chihuahua. Pictured here are Oak Trees (*Quercus* sp.) and Agave sp. Photo by Laura I. Heredia-González.

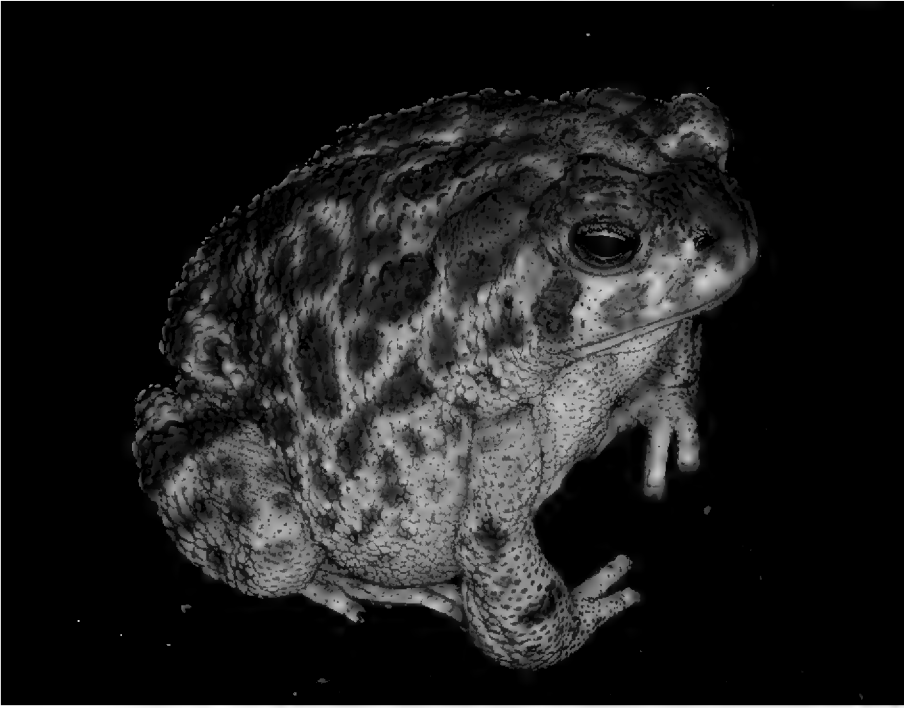
Cuauhtémoc valley. The plains along the edges of this valley are associated with soft hills, and occasionally with plateaus. Historically, the geologic evolution in this area has allowed for the development of an endorheic basin within the normal northwest to southeast fault pattern. Flooding is common in these areas, such as in the Bustillos and Los Mexicanos lagoons, of which the latter is the most significant (INEGI 1999).

According to INEGI (2003), this subprovince is located entirely within the state and covers 10.5% of the state's surface area, and it includes the following municipalities: Bachíniva, Namiquipa, Riva Palacio, Cuauhtémoc, and portions of Buenaventura, Carichí, Cusiuriachi, Chihuahua, Galeana, Santa Isabel, Gómez Farías, Gran Morelos, Guerrero, Ignacio Zaragoza, Madera, Matachí, and Temósachi. The headwaters of the Papigóchic and Santa María rivers and some tributaries of the Conchos River are located in this region. This subprovince includes three groups of north–south oriented ranges in the Sierra Tarahumara, the Sierra La Montosa-Las Tunas-El Rosal, and the Sierra del Nido. The elevations in this region range from 2,400 to 2,700 m. The terrain is composed predominately of acidic volcanic rocks, but to the west basaltic rocks dominate. The valley is composed of old conglomeratic alluvium.

Grasslands in this subprovince are dominated by *Bouteloua gracilis* and *Muhlenbergia rigida* (Estrada et al. 1997). In the highest mountains, especially in Cumbres de Majalca National Park, temperate vegetation such as pine, oak, and derivative forest are predominant and include *Pinus leiophylla* var. *chihuahuana*, *P. cembroides*, *P. engelmannii*, *Cupressus arizonica*, *Fraxinus velutina*, *Quercus hypoleucoides*, *Q. rugosa*, and *Q. grisea* (Estrada et al. 2003). In a recent study conducted by Vega-Mares et al. (2020) in La Sierra Azules, 30 km SW of the city of Chihuahua, 742 taxa in 353 genera were documented, with approximately 60% of the flora affiliated with the desert region, and the remainder in the temperate mountains.

Sierras y Llanuras de Durango (SLD). Located in the south-central part of the state, this subprovince (Fig. 4) is shaped like a narrow band with a north to south orientation. The elevations range from 1,300 to 2,360 m (Sierra La Boca). The prominent topoforms are valleys and slopes associated with smaller hills, and sometimes with isolated sierras of limited elevation (INEGI 1999). According to INEGI (2003), this subprovince lies on the eastern side of the SMO, covers 5.4% of the state's surface, and includes the following municipalities: Huejotitán, San Francisco del Oro, and Santa Bárbara, and parts of Allende, Balleza, Coronado, Cuauhtémoc, Cusiuriachi, Chihuahua, Doctor Belisario Domínguez, Santa Isabel, Gran Morelos, Hidalgo del Parral, Matamoros, Rosales, Rosario, Satevó, Saucillo, El Tule, and Valle de Zaragoza. The relief in this region consists of chains of small mountains, plateaus, and hills, but plains at elevations of 1,500 m and peaks that exceed 2,000 m also are present. The terrain is characterized by limestone rocks, shales, and slates, although acid igneous rocks predominate, and there are occasional outcrops of tertiary conglomeratic alluvium. Herbaceous vegetation predominates, and mostly consists of grasses. *Bouteloua gracilis* and *B. curtipendula* are abundant in non-disturbed areas. Trees only occur along streams and on the base of the mountains. The most common are *Juniperus* spp., which sometimes are dominant, as well as *Pinus cembroides* and oaks, principally *Quercus grisea*, *Q. emoryi*, and *Q. chihuahuensis* (González-Elizondo et al. 2007).

Gran Meseta y Cañones Chihuahuenses (GMCC). This subprovince (Fig. 5) is characterized by high sierras with well-defined canyons, like those found in Barrancas del Cobre, and contains extensive plateaus where canyons and intermountain valleys have formed. According to INEGI (1999), the high elevations in this region range from 500–2,740 m (Cerro Guichique). The GMCC covers 18.1% of the state's surface, is located in the southwestern portion of the state, and includes the municipalities of Batopilas, Bocoyna, Chínipas, Guachochi, Guazapares,



No. 1. *Anaxyrus cognatus* (Say, 1822). The distribution of the Great Plains Toad “extends throughout the Great Plains of the United States and extreme southern Canada, and southward in the Great Basin west of the Rocky Mountains from southern Utah to extreme northern Sinaloa, and east of the Sierra Madre Occidental nearly to the Transvolcanic Belt of south-central Mexico” (Lemos-Espinal and Dixon 2013: 34–35). This individual was photographed in scrub vegetation near the city of Chihuahua. Wilson et al. (2013b) calculated its EVS as 9, placing it at the upper level of the low vulnerability category. We assessed the conservation status of this toad as Least Concern, but this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 2. *Anaxyrus debilis* (Girard, 1854). The distribution of the Green Toad “extends from western Kansas and southeastern Colorado through southern New Mexico and western Texas, in the United States, and into much of the central plateau of Mexico” (Lemos-Espinal and Dixon 2013: 35–36). The image of this individual was taken in spiny shrub vegetation in Aldama, Chihuahua. Wilson et al. (2013b) calculated its EVS as 7, placing it in the middle portion of the low vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species was placed in the Special Protection category by SEMARNAT. *Photo by Sebastian Ochoa Rodríguez.*



No. 3. *Anaxyrus mexicanus* (Brocchi, 1879). The Mexican Spadefoot Toad occurs in “pine-oak and pine forests of the Sierra Madre [Occidental] of eastern Sonora and eastern Chihuahua, south to southwestern Durango and adjacent Sinaloa, Mexico” (Frost 2023). This individual was photographed on a rock in pine forest vegetation in Guachochi, Chihuahua. Wilson et al. (2013b) determined its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN status of this toad currently is listed as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 4. *Anaxyrus punctatus* (Baird and Girard, 1852). The distribution of the Red-spotted Toad “extends throughout the arid and semiarid areas of the southwestern United States, and in Mexico it occurs throughout Baja California and the Pacific slopes into Sinaloa, and across the Mexican Plateau as far south as Jalisco and Hidalgo (Lemos-Espinal and Dixon 2013: 37–38). This individual was photographed on a rock with associated microphyllous vegetation in the city of Chihuahua, Chihuahua. Wilson et al. (2013b) calculated its EVS as 5, placing it in the middle portion of the low vulnerability category. The conservation status of this toad has been determined as Least Concern by IUCN, and this species is not listed by SEMARNAT. *Photo by Sebastian Ochoa Rodríguez.*

Maguarichi, Nonoava, Urique, and Uruachi, as well as large portions of the municipalities of Carichí, Ocampo, and San Francisco de Borja, and small portions of Balleza, Cusihiuriachi, Guadalupe and Calvo, Guerrero, Doctor Belisario Domínguez, Morelos, Moris, Rosario, Satevó, Temósachi, and El Tule. Large plateaus in this subprovince are interrupted by deep canyons, including Barrancas del Cobre, which is 55 km long and contains pronounced peaks through which the Urique River crosses. The headwaters of this river are in the east-central portion of the province, and the Fuerte River also flows through this region. The average elevation is 2,200 m, although the highest areas are located in the eastern half of the subprovince, and range from 2,700 to 3,000 m. Notably, Cerro Las Iglesias attains an elevation of 3,100 m. The terrain is dominated by ignimbrite rocks with some basaltic outcrops (INEGI 2003). The vegetation of Barrancas del Cobre includes oak forest in the higher elevations (> 2,000 m), where trees such as *Quercus arizonica*, *Q. toumeyi*, *Q. oblongifolia*, *Pinus leiophylla*, and *P. engelmannii*, are predominant; the huizaches shrubs include *Acacia farnesiana* (250–300 m), which are associated with *Acacia cochliacantha*, *Ipomoea chilopsidis*, *Randia thurberi*, *Rhus tepetate*, and *Quercus chihuahuensis*, among others. The mauto *Lysiloma divaricatum* forest is formed by an association of trees and shrubs adapted to high temperatures, and includes *Lysiloma wootonii*, *Ceiba pentandra*, *Guazuma ulmifolia*, *Senna atomaria*, *Ipomoea arborescens*, *Caesalpinia platyloba*, *Pachycereus pectin-arboriginum*, *Stenocereus thurberi*, *Bursera lancifolia*, *B. pinicellata*, *B. grandiflora*, *Cordia sonora*, *Buddleja marrubifolia*, *Quercus albocincta*, *Q. tuberculata*, *Prosopis palmeri*, *P. articulata*, *Chloroleucon mangense*, and *Fouquieria mcdougallii*. Continuing to the fig river forest are species such as *Ficus petiolaris*, *F. pertusa*, and *F. conitifolia*, guamuchil *Pithecellobium dulce*, and tempisque *Sideroxylon tepicense*, which grow along the edges of the Batopilas and Urique rivers, and are joined with other species such as *Brongniartia alamosana*, *Coccoloba goldmanii*, *Tabernaia chrysantha*, *T. Impetiginosa*, *Platimiscium trifoliolatum*, *Vitex mollis*, and *V. pyramidata* (Lebgue et al. 2005). The predominant vegetation types are pine forest, oak forest, and combinations of these species with alamillo, and important species like *Picea chihuahuana*, *Pseudotsuga* spp., and *Abies concolor* (WWF 2008). In a herpetological study in Chínipas Canyon, Chihuahua, Santoyo-Brito and Lemos-Espinal (2010) characterized three different plant associations in the lower level (200–1,200 m) of this region, which includes tropical deciduous forest composed of *Bursera fagaroides*, *Guazuma ulmifolia*, *Ipomoea arborescens*, and *Lysiloma divaricata*; oak forest at elevations from 1,200–1,750 m, with *Quercus chihuahuensis* at the higher elevations (1,750–2,500 m); and pine forest with *Pinus ayacahuite*, *P. durangensis*, *P. engelmannii*, and *P. leiophylla*.

Gran Meseta y Cañadas Duranguenses (GMCD). Most of this subprovince is characterized by high elevations associated with canyons, and plateaus with glens (Fig. 6). According to INEGI (1999), the elevations range from 700 to 3,300 m (Cerro Mohinora). Located to the south of the municipality of Guadalupe y Calvo and Morelos, this subprovince covers 2.1% of the surface area of Chihuahua,

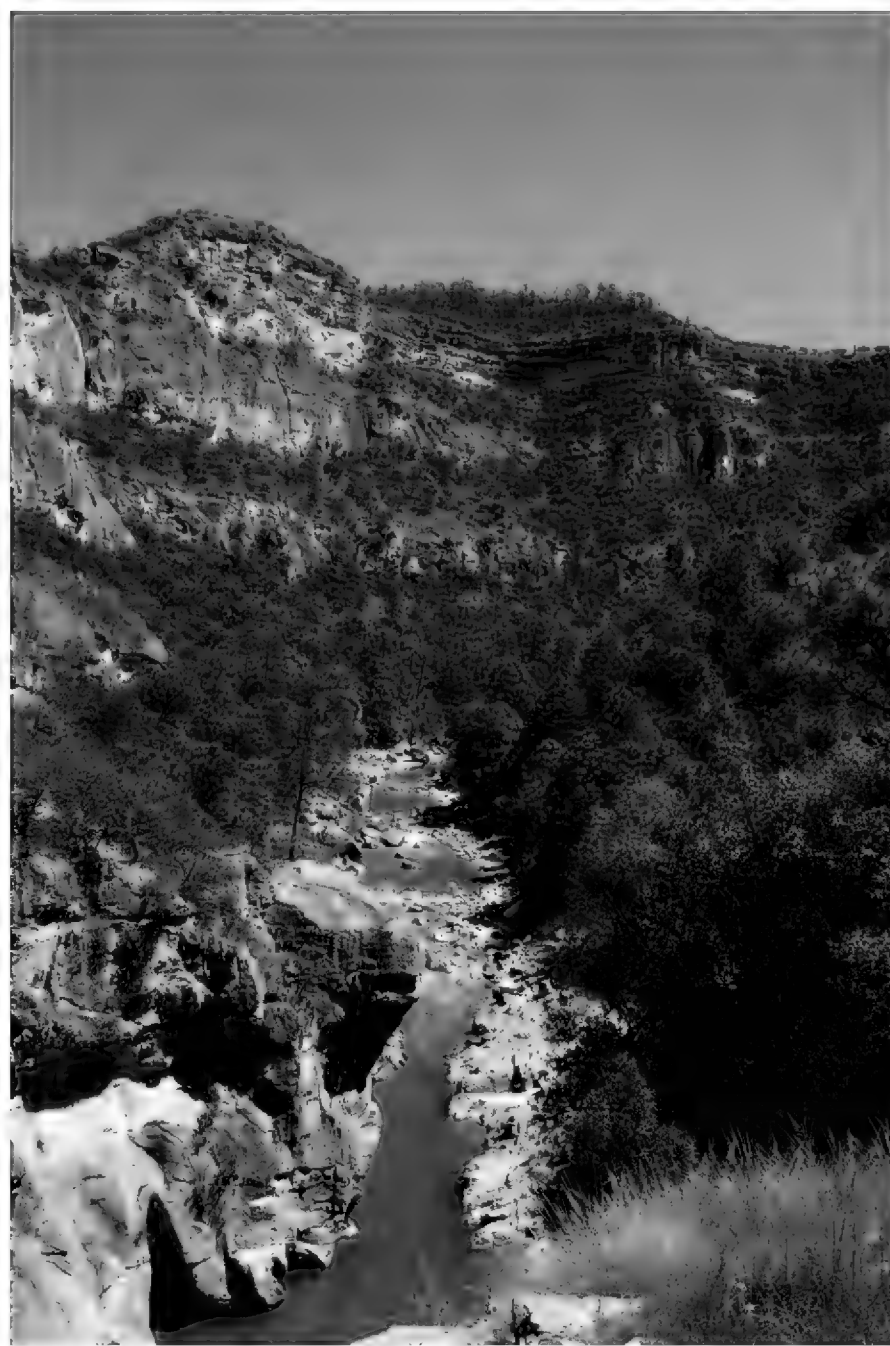


Fig. 5. Gran Meseta y Cañones Chihuahuenses (GMCC). Rio Urique, north of Huasaronare, Guachochi, Chihuahua, within pine and gallery forest. Photo by Jesús M. Martínez-Calderas.

and thus is the smallest in the state. The plateau is greatly dissected where the canyon rivers flow, and basaltic and intermediate rocks dominate the terrain (INEGI 2003). A study by García-García et al. (2019) reported the diversity and vertical structure (three levels) of a pine-oak forest in Guadalupe y Calvo by examining the phytosociological relative value in a managed forest. That study determined the common species as *Pinus durangensis*, *Quercus sideroxyla*, *Q. fulva*, *Pinus arizonica*, and *Arbutus xalapensis*, and demonstrated their relative presence in the levels as *Pinus durangensis*, *Q. fulva*, *Arbutus xalapensis*, *Quercus sideroxyla*, and *P. arizonica*. According to Martínez (1991), the vegetation is dominated and conditioned by elevation, with the presence of coniferous forest and a small belt of subalpine vegetation near the summit.

Sierras y Llanuras del Norte (SLN) and its four subprovinces

This province (Fig. 1) extends from the border of the state of Texas (USA) to near Nazas de Durango and encompasses the arid region of the state. The Chihuahuan Desert has been delimited by various authors, including Morafka (1977) who defined it based on the distribution of amphibians and reptiles, highlighting the Pleistocene isolation and divergence between the Chihuahuan and Sonoran deserts. He considered evidence of vicariant events ranging from the late Neogene to the late



Fig. 6. Gran Meseta y Cañadas Duranguenses (GMCD). The summit of Cerro Mohinora (3,300 m), located in a Natural Protected Area in the municipality of Guadalupe y Calvo, Chihuahua. *Photo by César F. Hernández-Urbina.*



Fig. 7. Llanuras y Médanos del Norte (LMN). The core area of Médanos de Samalayuca, a Natural Protected Area in Juárez, Chihuahua. Sierra Presidio lies in the background. *Photo by Jesús M. Martínez-Calderas.*

Pleistocene, which separated the herpetofauna of the western continental deserts (Sonoran, Sinaloan, and Mohave) and the eastern (Chihuahuan) along the Sierra Madre Occidental.

The SLN is made up of abrupt mountains that rise to 3,000 m and plains with elevations of 800–1,000 m. This province covers 56.5% of the surface area of the state. The dominant climates are very dry, semi-warm, and temperate, but a region of temperate semi-dry climate lies next to the SMO (INEGI 2003). The four subprovinces in this region are the Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Del Bolsón de Mapimí (BDM), and Llanuras y Sierras Volcánicas (LSV).

Llanuras y Médanos del Norte (LMN). This subprovince (Fig. 7) is located in northwestern Chihuahua, where the dominant topoforms include folded and steep mountain ranges, oriented in various directions (e.g., northwest to southeast). The elevations of this topoform range from 2,300 to 2,500 m (Cerro Grande and Sierra La Escondida). The most common associations are slopes and hills, which define the floodplains, and the terrain is occasionally rocky or hard

but generally salty and subject to flooding. The city of Samalayuca stands out for its distinctiveness (INEGI 1999). According to INEGI (2003), this subprovince borders the USA to the north and Sonora to the west, and covers 17.4% of the state's surface area. It includes the municipalities of Ascensión and Nuevo Casas Grandes, and parts of Ahumada, Buenaventura, Casas Grandes, Janos, Galeana, and Juárez. The Casas Grandes and Santa María rivers penetrate the southern portion of the subprovince and terminate in this region. Here, the plains have wide slopes, small but steep mountain ranges, and dune fields, and the average elevation is about 1,000 m. The terrain is characterized by alluvium, limestone, and acidic volcanic rocks that compose the mountains and hills, as well as basaltic rocks that are present in the middle of the subprovince. In a study conducted in the Janos-Casas Grandes Prairie Dog Complex in the extreme northwestern Chihuahua, Ceballos et al. (2010) indicated a mosaic of native grasslands and shrublands. The grasslands are dominated by the annual grasses, including Sixweeks Threawn (*Aristida adscensionis*), Needle Grama (*Bouteloua aristidoies*), and Sixweeks Grama (*B. barbata*), and numerous forbs; perennial



Fig. 8. Sierras Plegadas del Norte (SPN). Southwest of Sierra Presidio, in the municipality of Guadalupe, Chihuahua. Note the typical rosettophyllous vegetation. *Photo by Ana Gatica-Colima.*



Fig. 9. Del Bolsón de Mapimí (BDM). A scene showing water from the Conchos River flowing to Rosetilla Dam, Saucillo, Chihuahua. *Photo by Ana Gatica-Colima.*

grasses include Poverty Treeawn (*Aristida divaricate*), Ear Muhly (*Muhlenbergia arenacea*), Burrograss (*Scleropogon brevifolius*), Vine Mesquite (*Panicum obtusum*), Tobosagrass (*Pleuraphis mutica*), Blue Grama (*Bouteloua gracilis*), Black Grama (*B. eripoda*), and Red Grama (*B. trifida*); and the shrublands are dominated by mesquite, ephedra, and Cholla (*Opuntia imbricata*). Quiñonez-Martínez et al. (2018) reported that the vegetation in Ejido Villa Luz at the Médanos de Samalayuca Natural Protected Area is characterized by xeric scrub, with dominant shrubs such as *Larrea tridentata* and *Prosopis juliflora* var. *glandular*. They also indicated some lesser abundant species such as *Atriplex canescens*, *Fouquieria splendens*, *Flourensia cernua*, and *Yucca* sp.; additionally, some dunes are devoid of vegetation, while others contain shrubs such as *Prosopis juliflora* var. *glandulosa*, *Atriplex canescens*, *Poliomintha incana*, and *Artemisia filifolia*, as well as some Cactaceae.

Sierras Plegadas del Norte (SPN). This subprovince (Fig. 8) is located in northeastern Chihuahua, and is oriented in a north to south direction. It contains

different types of topoforms, including elongated sierras with a northwest to southeast orientation, which contain anticlinal structures of folded rocks composed of carbonate that date back to the Cretaceous. Occasionally, these topoforms are associated with hills and plateaus, and many of the hills are steep and branched. The plains are located in desert areas, and have developed on alluvial fills. Salty terrain is associated with the hills and slopes, which sometimes becomes flooded because of its development on rocky terrain (INEGI 1999).

This subprovince contains 13.6% of the state's surface area, and is located in the north-central portion of the state. It includes the municipalities of Praxedis G. Guerrero, and parts of Ahumada, Aldama, Coyame, Guadalupe, Juárez and Julimes. The slopes are associated with hills that are interrupted by small and elongated mountain ranges, with plains located near the capital of Juárez. In general, the elevation in this area is about 1,000 m. The terrain is rocky and characterized by desert plains with rocky terrain, but in Samalayuca it becomes more saline and sometimes floods (INEGI 2003).

Macias-Duarte et al. (2004) studied the Aplomado Falcon (*Falco femoralis*) in two areas: Sueco (in the



Fig. 10. Llanuras y Sierras Volcánicas (LSV). A Creosote Bush (*Larrea tridentata*), photographed about 5.8 mi from Benito Juárez, Manuel Benavides, Chihuahua. The higher elevations of Santa Elena Canyon are seen in the background. *Photo by Ana Gatica-Colima.*

municipalities of Ahumada and Chihuahua) and Tinaja Verde (in the municipality of Coyame). The vegetation in these areas consists of open grasslands dominated by *Bouteloua gracilis*, *B. hirsuta*, and *B. eriopoda*, with *B. curtipendula*, *Aristida* spp., *Hilaria mutica*, and *Enneapogon desvauxii* as subdominant species. These grasslands are invaded by woody species, with *Acacia constricta*, *Ephedra trifurca*, *Flourensia cernua*, and *Larrea tridentata* being the most prominent. Additionally, open halophytic grasslands typically occur in swales and are characterized by *Hilaria mutica* and *Sporobolus airoides* and other grasses, such as *Bouteloua gracilis* and *Panicum obtusum*. The most common woody species are *Prosopis glandulosa*, *Koeberlinia spinosa*, *Flourensia cernua*, *Acacia constricta*, and *Condalia ericoides*.

Bolsón de Mapimí (BDM). Located in the central part of the state, this subprovince (Fig. 9) consists of an elongated band with a northwest to southeast orientation. Structurally, the topoforms in the sierras are oriented from northwest to southeast and west to east, and contain anticlines with carbonite rocks that developed in the Cretaceous. A panoramic view reveals a steep relief that often is associated with plateaus, hills, slopes, and ravines. The hills are steep and branched, and often are associated with slopes. Plains and ravines are found throughout the south-central portion of this subprovince, and the slopes of the broadest hillsides take up much of the terrain in the north-central part of this region. The plains in this region developed on alluvial soil in desert areas with a saline presence, which was deposited in rocky ground that can flood. The least distributed topoforms are plateaus related to basalt flows, which gradually transformed into hills, and the scenery is dominated by “malpaís” and dunes (INEGI 1999).

This subprovince represents 12.4% of the state’s surface, and it is located southeast of the Sierras Plegadas del Norte and Llanuras y Sierras Volcánicas subprovinces. It extends over the territory of Aquiles Serdán, Delicias, López, Meoqui, and San Francisco de Conchos, and

parts of the municipalities of Ahumada, Aldama, Allende, Buenaventura, Camargo, Coronado, La Cruz, Chihuahua, Hidalgo de Parral, Jiménez, Julimes, Matamoros, Rosales, Satevó, Saucillo, and Valle de Zaragoza. The land primarily is composed of alluvial plains and lowlands, but also contains steep and folded mountains and steep and branched hills. The average elevation is 1,200 m. The terrain is characterized by the presence of acid basaltic rocks and limestone hills. Northeast of Hidalgo del Parral, the morphology of the rocks resembles that of a plateau (INEGI 2003).

The vascular flora of central Chihuahua primarily is composed of eight biological forms: herbs, inerns, shrubs, trees, vines, crassicaules, thorny shrubs, rosetophyllous shrubs, and parasites. Herbaceous species predominate over the remainder of the area. In scrub communities with plains and hills, the more densely occurring species are *Larrea tridentata*, *Acacia neovernicosa*, *Flourensia cernua*, *Parthenium confertum*, and *Parthenium incanum* (Estrada-Castillón and Villarreal-Quintanilla 2010).

Moreno-Contreras et al. (2021) worked in the riverine area of Meoqui, where the vegetation consists of gallery forest and includes trees such as cottonwood *Populus* sp., willow *Salix* sp., and ash *Fraxinus* sp., as well as cattail *Typha* sp., with Bulrush (*Schoenoplectus americanus*) in low places and along the edge of the river, and desert thickets of mesquite *Prosopis* sp. and agave *Agave* sp. are present in the drier open areas.

Llanuras y Sierras Volcánicas (LSV). The topoforms in the sierras (Fig. 10) are oriented in a northwest to southeast direction and consist of anticlinal products of carbonated sequences of folding that date back to the Cretaceous. Part of its appearance consists of steep and branched hills and plateaus that in some associations contain distinctive ravines and slopes. Slopes are the most abundant, especially because of their relationship with hills, and the plains occur on alluvial grounds on rocky or granular terrain, as well as dunes with salinity where flooding is common (INEGI 1999).

The herpetofauna of Chihuahua, Mexico



No. 5. *Anaxyrus woodhousii* (Girard, 1854). Woodhouse's Toad is found in the "Western USA (excluding Great Basin and Pacific Coast) and northern Mexico (northeastern Sonora to north-eastern Coahuila south to eastern Durango)" (Frost 2023). This individual was photographed along a road on a rainy night near Santa Eulalia, Chihuahua. Wilson et al. (2013b) determined its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN indicated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ramón Isaac Miramontes Cinco.*



No. 6. *Incilius mazatlanensis* (Taylor, 1940). The distribution of the Sinaloa Toad extends from "Northern Sonora from the municipality of Imuris and southwestern Chihuahua (Río Fuerte drainage) to Colima, along the Pacific coastal plain, Mexico; reported on the Pacific drainage of Durango and Jalisco, Mexico; reported in the Pacific drainage of Durango and Jalisco, Mexico; Isla Maria Madre, Nayarit, Mexico" (Frost 2023). This individual was photographed in lowland deciduous forest in Guapalaina, in the municipality of Urique, Chihuahua. Wilson et al. (2013b) determined its EVS as 12, placing it in the upper portion of the medium vulnerability category. The IUCN determined its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Javier Cruz-Nieto.*



No. 7. *Dryophytes arenicolor* (Cope, 1886). The distribution of the Canyon Treefrog "extends from the mountains of southern Colorado, western New Mexico, and southern Arizona, in the United States, southward to Guerrero and northern Oaxaca in Mexico; isolated populations, however, are found in the Big Bend area of Texas, adjacent Coahuila, and in San Luis Potosí" (Lemos-Espinal and Dixon 2013: 52–53). This individual was photographed in pine forest at Monterde, in the municipality of Guazapares, Chihuahua. Wilson et al. (2013b) calculated its EVS as 7, placing it in the middle portion of the low vulnerability category. The IUCN assessed its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 8. *Dryophytes wrightorum* (Taylor, 1939). The Mountain Treefrog occurs at "discontinuous distributions generally above 2,000 m in the Mogollon Rim of central Arizona and west-central New Mexico, the Huachuca Mountains area (at elevations down to about 1,400 m) in southeastern Arizona, and Peloncillo Mountains of Hidalgo County, New Mexico, and southward in grama grasslands and pine-oak parklands in the Sierra Madre Occidental and south to, but not including, the state of México, Mexico" (Frost 2023). This individual was photographed in pine forest at Guachochi, Chihuahua. Wilson et al. (2013b) assessed its EVS as 9, placing it at the upper limit of the low vulnerability category. The IUCN assessed its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*

Table 1. Monthly minimum, mean (in parentheses \pm SD), maximum, and annual temperature data (in $^{\circ}$ C) for the nine physiographic regions of Chihuahua, Mexico. Data were obtained from Normales Climatológicas 1950–2010 CONAGUA-SMN.

Physiographic region	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Llanuras y Médanos del Norte	-0.9 (10.9 \pm 3.0) 15.8	0.5 (12.9 \pm 2.6) 18.4	3.1 (16.0 \pm 2.7) 21.8	6.5 (19.9 \pm 2.5) 25.8	10.3 (23.8 \pm 2.6) 30.0	14.7 (27.9 \pm 2.9) 34.2	16.7 (28.0 \pm 2.8) 33.0	15.9 (27.0 \pm 2.7) 31.9	13.4 (25.0 \pm 2.3) 29.7	7.8 (20.2 \pm 2.6) 25.4	1.9 (14.1 \pm 2.6) 19.8	-0.8 (11.0 \pm 2.7) 15.9	7.5 (19.8 \pm 2.7) 25.3
Sierras Plegadas del Norte	-0.6 (8.9 \pm 5.5) 17.7	1.0 (11.3 \pm 1.8) 20.5	4.8 (15.2 \pm 1.9) 24.7	9.3 (19.9 \pm 1.8) 29.6	13.7 (24.0 \pm 1.6) 33.5	17.8 (27.9 \pm 1.7) 36.9	18.5 (27.8 \pm 1.6) 35.6	17.9 (26.9 \pm 1.5) 34.6	14.7 (23.8 \pm 2.0) 32.3	9.8 (19.3 \pm 1.6) 27.8	3.4 (13.3 \pm 1.9) 22.4	-1.6 (9.4 \pm 1.7) 18.3	6.9 (18.4 \pm 2.6) 27.2
Bolsón de Mapimí	0.5 (10.9 \pm 1.8) 20.0	2.7 (14.6 \pm 1.8) 22.6	5.6 (16.5 \pm 1.6) 26.5	9.7 (19.7 \pm 1.8) 30.3	14.4 (22.3 \pm 1.5) 33.7	18.0 (24.5 \pm 1.6) 35.7	18.1 (23.7 \pm 1.7) 33.7	17.4 (22.7 \pm 5.3) 32.7	15.2 (21.3 \pm 1.7) 30.8	10.32 (18.7 \pm 1.7) 28.5	4.2 (15.2 \pm 1.7) 24.0	1.4 (12.6 \pm 1.6) 20.6	5.7 (17.8 \pm 1.7) 28.1
Llanuras y Sierras Volcánicas	-1.4 (8.3 \pm 1.6) 18.1	0.7 (10.8 \pm 2.2) 20.9	3.4 (14.2 \pm 1.7) 25.0	7.0 (19.3 \pm 1.7) 29.6	11.1 (23.5 \pm 1.5) 33.9	15.6 (27.9 \pm 2.2) 37.4	16.9 (26.3 \pm 2.7) 36.4	16.0 (25.5 \pm 2.7) 34.8	13.3 (22.7 \pm 2.0) 32.1	7.7 (19.0 \pm 1.4) 28.4	2.6 (12.4 \pm 1.6) 22.8	-0.8 (8.6 \pm 1.7) 18.1	8.0 (19.5 \pm 1.6) 28.4
Sierras y Cañadas del Norte	-7.9 (4.9 \pm 1.4) 13.7	-6.6 (6.1 \pm 1.7) 15.0	-4.8 (8.4 \pm 1.5) 18.3	-1.5 (11.8 \pm 1.5) 21.7	1.9 (15.1 \pm 1.2) 24.0	6.5 (19.2 \pm 1.5) 27.7	10 (19.5 \pm 1.1) 25.8	9.2 (18.7 \pm 1.0) 25.4	6.5 (16.9 \pm 1.3) 24.5	0.2 (12.8 \pm 1.3) 22.6	-4.7 (8 \pm 1.8) 17.9	-7.5 (5.2 \pm 1.5) 14.1	-0.9 (12.2 \pm 1.7) 21.0
Sierras y Llanuras Tarahumaras	-4.7 (6.7 \pm 1.5) 15.5	-3.4 (8.3 \pm 1.5) 17.6	-1.2 (10.9 \pm 1.5) 20.4	1.9 (14.3 \pm 1.5) 24.1	4.7 (18.1 \pm 2.0) 28.0	9.9 (22.0 \pm 1.5) 31.4	12.4 (21.4 \pm 1.6) 28.8	11.6 (20.5 \pm 1.3) 27.8	9.0 (18.8 \pm 4.2) 26.4	3.1 (15.0 \pm 1.2) 24.0	-2.1 (10.2 \pm 1.4) 19.8	-4.5 (8.7 \pm 1.6) 16.3	0.8 (14.7 \pm 6.4) 24.2
Sierras y Llanuras de Durango	0.2 (9.4 \pm 2.3) 18.6	1.5 (11.2 \pm 1.8) 20.9	4.7 (14.3 \pm 1.5) 24.0	8.0 (17.8 \pm 1.5) 27.6	11.8 (21.4 \pm 1.5) 31.1	15.9 (24.9 \pm 1.4) 33.8	16.1 (23.9 \pm 1.4) 31.5	15.4 (23.0 \pm 1.3) 30.4	13.8 (21.2 \pm 1.4) 28.6	9.4 (18.0 \pm 1.6) 26.6	3.6 (13.1 \pm 1.7) 22.7	0.7 (10.2 \pm 1.8) 19.7	8.4 (17.4 \pm 1.6) 26.3
Gran Meseta y Cañones Chihuahuenses	-0.1 (12.5 \pm 1.6) 21.6	0.7 (13.9 \pm 1.7) 23.4	2.7 (16.1 \pm 1.6) 25.8	5.1 (19.1 \pm 1.5) 29.0	9.3 (22.4 \pm 1.5) 32.4	13.0 (25.7 \pm 1.5) 34.6	14.3 (24.7 \pm 1.4) 31.8	13.7 (24.1 \pm 1.3) 31.0	12.6 (23.1 \pm 2.1) 30.4	6.5 (19.8 \pm 1.5) 28.4	3.0 (15.3 \pm 1.6) 25.2	-1.9 (13.5 \pm 1.6) 22.0	5.4 (19.4 \pm 2.8) 28.1
Gran meseta y Cañones Duranguenses	-1.7 (8.7 \pm 1.3) 19.2	-2.0 (9.2 \pm 1.7) 20.4	-0.4 (11.1 \pm 1.8) 22.6	1.9 (13.7 \pm 1.5) 25.5	4.4 (16.2 \pm 1.0) 28.0	8.8 (19.1 \pm 1.7) 29.4	10.5 (18.9 \pm 1.0) 27.3	10.4 (18.9 \pm 1.0) 27.5	9.5 (18.2 \pm 1.3) 27.0	4.9 (15.5 \pm 1.2) 26.1	0.2 (11.6 \pm 1.8) 22.9	-1.0 (9.7 \pm 1.3) 20.3	3.8 (14.2 \pm 1.4) 24.7

This subprovince comprises 13.2% of the state’s surface area, is located in the southeastern portion of the state, and includes the municipalities of Manuel Benavides and portions of Camargo, La Cruz, Jiménez, Julimes, Ojinaga, and Saucillo. The relief is characterized by plains, slopes, and flattened surfaces that sometimes are interrupted by mountains, hills, or plateaus. The mountains generally are composed of volcanic rock, but small mountain ranges also occur, while the plains are alluvial and those with salinity sometimes are flooded (INEGI 2003).

González and Sosa (2003) identified nine vegetational groups in the natural protected area of Cañón de Santa Elena, based on elevation and botanical composition. At the lowest elevations, level 1 (600–900 m), the vegetation primarily is composed of *Larrea tridentata*, *Jatropha dioica*, and *Prosopis glandulosa*; in level 5 (1,300–1,600 m) it contains *Vachellia constricta*, *Viguiera stenoloba*, and *Mimosa wherryana*; and in level 9 (2,300–2,400 m) it consists of *Muhlenbergia monticola*, *Pinus cembroides*, *Quercus grisea*, and *Bouteloua gracilis*.

Climate

Temperature. In Table 1, we provide the monthly minimum, maximum, and mean annual temperatures for each of the nine physiographic regions recognized in Chihuahua. The highest mean annual temperature is in the Llanuras y Médanos del Norte at 19.8 $^{\circ}$ C, and the lowest is in the Sierras y Cañadas del Norte, at 12.2 $^{\circ}$ C. The mean monthly temperatures generally are highest in June and lowest in January. The differences between the monthly minimum and maximum temperatures in the nine regions range from 17.8 $^{\circ}$ C to 22.7 $^{\circ}$ C.

Precipitation. The precipitation regime (Figs. 11–12) in the nine physiographic regions of Chihuahua typically consists of a 5-month “rainy” season that extends from June to October, and a 7-month “dry” season that lasts from November to May (Table 2). The mean monthly rainfall generally is highest in July or August. The mean annual precipitation ranges from 196.8 mm in the Llanuras y Sierras Volcánicas to 922.3 mm in the Gran Meseta y Cañones Duranguenses.

Composition of the Herpetofauna

Families. The species of herpetofauna in Chihuahua are classified into 34 families, including eight families of anurans, two of caudates, 19 of squamates, and five of turtles (Table 3). No families of caecilians or crocodylians are represented within the state. The total of 34 families makes up 56.7% of the 60 families represented in all of Mexico (J. Johnson, unpublished data, 27 May 2023). Among the 10 families of amphibians represented in Chihuahua (Table 4), 50.0% (20) of the 40 species are allocated to the families Bufonidae (11 species) and Ranidae (nine species). Among the 24 families of reptiles, 79.5% (116) of the 146 species are assigned to the families Phrynosomatidae (23 species), Scincidae (seven species), Teiidae (nine species), Colubridae (40 species), Dipsadidae (10 species), Natricidae (11 species), Viperidae (11 species), and Kinosternidae (five species).

The herpetofauna of Chihuahua, Mexico



Fig. 11. A scene from the rainy season during the month of August in the municipality of Ahumada, Chihuahua. Sierra Candelaria is seen in the background. *Photo by Ana Gatica-Colima.*



Fig. 12. The snowy season in December at Rancho El Uno in Cerro de la Cal, a Biosphere Reserve located in Janos, Chihuahua. *Photo by Laura I. Heredia-González.*

Genera. Eighty-two genera of amphibians and reptiles are represented in Chihuahua, including 14 genera of anurans, two of salamanders, 59 of squamates, and seven of turtles (Table 3). These 82 genera constitute 38.0% of the 216 genera known from Mexico (J. Johnson, unpublished data, 27 May 2023). Among the anurans and salamanders (Table 4), the most speciose genera are *Anaxyrus* (six species), *Lithobates* (nine species), and *Ambystoma* (four species). Among the reptiles (Table 4), the most speciose genera are *Phrynosoma* (four species), *Sceloporus* (11 species), *Plestiodon* (seven species), *Aspidoscelis* (nine species), *Lampropeltis* (four species), *Masticophis* (four species), *Salvadora* (four species), *Tantilla* (six species), *Rena* (four species), *Thamnophis* (nine species), *Crotalus* (nine species), and *Kinosternon* (five species).

Species. The herpetofauna of Chihuahua is composed of 186 species, including 35 species of anurans, five of salamanders, 133 of squamates, and 13 of turtles (Table 3). Of these 186 species, 183 are native to the state and three are non-native. Presently, the numbers of native species in these groups in Mexico are 272, 161, 913, and 51, respectively (J. Johnson, unpublished data, 27 May 2023). The 183 native species in Chihuahua constitute 13.0% of the 1,403 native species in all of Mexico (J. Johnson, unpublished data, 27 May 2023).

Patterns of Physiographic Distribution

We recognize nine physiographic regions in Chihuahua (Fig. 1). We document the distributions of the members of the herpetofauna among these nine regions in Table 4, and summarize the data in Table 5.

The numbers of species in the nine physiographic regions range from 58 in the Sierras y Llanuras de Durango to 128 in the Gran Meseta y Cañones Chihuahuenses. The values for the remaining seven regions, in ascending order are: 59 (Gran Meseta y Cañones Duranguenses); 61 (Sierras y Llanuras Tarahumaras); 64 (Sierras Plegadas del Norte); 75 (Llanuras y Sierras Volcánicas); 78 (Sierras y Cañadas del Norte); 80 (Bolsón de Mapimí); and 81 (Llanuras y Médanos del Norte). The mean figure for regional occupancy in Chihuahua is 76.0, or 40.9% of the state's total.

As expected, the greatest absolute and relative numbers of species are found among amphibians (30; 23.4%) and squamates (98; 76.6%) in the Gran Meseta y Cañones Chihuahuenses. Conversely, turtles are most prominently represented in the Llanuras y Médanos del Norte at nine species (11.1% of the regional total of 81).

Members of the herpetofauna of Chihuahua inhabit from one to nine physiographic regions (Table 4), as follows: one (42 of 186; 22.6%); two (32; 17.2%); three (27; 14.5%); four (28; 15.1%); five (16; 8.6%); six (12;



No. 9. *Lithobates berlandieri* (Baird, 1859). The distribution of the Rio Grande Leopard Frog “extends from New Mexico and Texas, in the United States, southward in Mexico through eastern Chihuahua, Coahuila, Nuevo León, Tamaulipas, Querétaro, and Hidalgo. It also occurs throughout the lowlands of Veracruz, except for the southern portion of the state and along the Transvolcanic Belt to Colima. Additionally, an isolated population is present in western Chihuahua and an introduced population in extreme northwestern Sonora” (Lemos-Espinal and Dixon 2013: 67–68). This individual was photographed in a creek at San Carlos canyon, a Natural Protected Area in Cañón de Santa Elena, in the municipality of Manuel Benavides, Chihuahua. Wilson et al. (2013b) determined its EVS as 7, placing it in the middle portion of the low vulnerability category. The IUCN established its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ana B. Gatica-Colima.*



No. 10. *Lithobates catesbeianus* (Shaw, 1802). The distribution of the American Bullfrog “extends from southeastern Canada and throughout most of the eastern and midwestern United States, although it has been introduced into several areas in the western part of the country. In Mexico, it occurs in the northeastern coastal plain” (Lemos-Espinal and Dixon 2013: 68–69). This individual was photographed in the San Pedro River wetland, located in Meoqui, Chihuahua. Wilson et al. (2013b) calculated its EVS as 10, placing it at the lower limit of the medium vulnerability category. The conservation status of this frog was determined as Least Concern by IUCN, and this species is not listed by SEMARNAT. *Photo by Leonardo Hernández-Escudero.*



No. 11. *Lithobates cora* (Pérez-Ramos and Luja-Molina, 2022). The Náayari Leopard Frog (as indicated in the original publication) occurs from “extreme southwestern Chihuahua, west-central Sonora, through Sinaloa, Nayarit, and Colima, Mexico, sea level to 1,250 m elevation; presumably penetrating into the barrancas of western Durango” (Frost 2023). This individual was photographed in lowland deciduous forest at Guapalaina, in the municipality of Urique, Chihuahua. The EVS of this frog was calculated as 13, placing it at the upper limit of the medium vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. *Photo by Javier Cruz-Nieto.*



No. 12. *Lithobates tarahumarae* (Boulenger, 1917). Previously, the Tarahumara Frog was known to occur “in extreme southern Arizona (USA, although now extinct there), south through the Sierra Madre Occidental of eastern Sonora, western Chihuahua, and eastern Sinaloa, likely in adjacent western Durango and northern Jalisco, Mexico; southwestern Aguascalientes” (Frost 2023). This individual was photographed in a river in the Sierra Tutuaca, in the municipality of Madera, Chihuahua. Wilson et al. (2013b) determined its EVS as 8, placing it in the upper portion of the low vulnerability category. The IUCN ascertained its conservation status as Vulnerable, but this species is not listed by SEMARNAT. *Photo by Juan Cruzado-Cortés.*

Table 2. Monthly minimum, mean (in parentheses \pm SD), maximum, and annual precipitation data (in mm) for nine physiographic regions of Chihuahua, Mexico. Data were obtained from: SMN (s/f) Servicio Meteorológico Nacional, Normales Climatológicas por Estado Chihuahua: <https://smn.conagua.gob.mx/es/informacion-climatologica-por-estado?estado=chih> (Accessed 24 November 2023). The shaded area indicates the months of the rainy season.

Physiographic region	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Llanuras y Médanos del Norte	0 (12.7 \pm 5.3) 62.1	0 (9.6 \pm 11.9) 52.3	0 (22.0 \pm 11.2) 55.2	0 (6.7 \pm 12.4) 67.0	0 (6.6 \pm 12.2) 65.1	0 (14.7 \pm 20.0) 80.7	4.9 (66.0 \pm 42.2) 182.6	6.7 (62.6 \pm 38.5) 150.4	0.5 (40.7 \pm 32.9) 125.7	0 (22.2 \pm 6.7) 119.7	0 (10.5 \pm 12.3) 50.7	0 (13.5 \pm 17.5) 76.1	3.6 (262.6 \pm 9.5) 519.1
Sierras Plegadas del Norte	0.9 (9.2 \pm 9.3) 39.1	1.3 (8.0 \pm 8.4) 47.2	2.6 (7.9 \pm 8.6) 46.5	4.1 (9.8 \pm 11.1) 59.6	5.2 (14.6 \pm 11.1) 47.1	5.9 (26.5 \pm 28.9) 110.3	6.9 (50.6 \pm 36.1) 174.7	6.8 (50.6 \pm 29.1) 125.2	3.1 (41.6 \pm 37.8) 173.4	3.5 (22.9 \pm 22.5) 107.0	1.8 (10.3 \pm 10.9) 55.9	0.7 (10.4 \pm 14.1) 71.9	3.6 (241.6 \pm 7.7) 442.7
Bolsón de Mapimi	0 (8.4 \pm 14.1) 67.2	0 (12.4 \pm 7.0) 31.2	0 (2.6 \pm 5.6) 26.9	0 (7.7 \pm 15.1) 68.4	0 (12.5 \pm 15.9) 61.9	0 (12.5 \pm 31.6) 125.4	1.1 (31.2 \pm 48.6) 238.1	3.7 (65.4 \pm 50.7) 260.4	0.3 (67.3 \pm 55.7) 236.4	0 (22.0 \pm 25.1) 96.4	0 (6.8 \pm 12.1) 67.1	0 (8.2 \pm 11.5) 43.9	4.5 (253.3 \pm 10.3) 596.8
Llanuras y Sierras Volcánicas	0 (4.7 \pm 8.6) 34.3	0 (4.1 \pm 9.1) 33.7	0 (3.1 \pm 6.5) 24	0 (4.6 \pm 8.0) 31.2	0 (9.0 \pm 9.7) 35.3	0 (21.4 \pm 27.4) 88.7	0.5 (38.3 \pm 38.0) 144.5	1.4 (42.5 \pm 42.6) 177.7	0.3 (42.4 \pm 46.5) 107.7	0 (21.5 \pm 26.5) 105.1	0.7 (6.4 \pm 11.3) 44	13.3 (6.5 \pm 11.5) 50.5	1.1 (196.8 \pm 11.0) 427
Sierras y Cañadas del Norte	0 (28.2 \pm 24.0) 101.1	0 (30.5 \pm 15.6) 87.5	0 (12.2 \pm 12.4) 87.8	0 (8.1 \pm 10.7) 61.3	0.5 (7.3 \pm 9.5) 61.4	25.9 (33.2 \pm 33.6) 137.7	10.5 (103.5 \pm 68.9) 263.8	0 (92.3 \pm 59.0) 260.4	0 (46.3 \pm 39.1) 161.6	0 (26.1 \pm 27.7) 138.6	0 (16.7 \pm 14.4) 79.4	0 (24.9 \pm 23.6) 122.0	9.2 (605.9 \pm 221.3) 847.0
Sierras y Llanuras Tarahumaras	0 (17.8 \pm 25.3) 103.1	0 (7.1 \pm 15.3) 90.3	0 (4.8 \pm 8.6) 36.8	0 (15.5 \pm 10.5) 50.4	0 (6.0 \pm 11.6) 57.7	0 (28.2 \pm 30.0) 137.8	0 (98.0 \pm 74.2) 399.5	0 (97.0 \pm 69.9) 320.38	0 (62.4 \pm 55.5) 246.3	0 (24.2 \pm 30.8) 143.1	0 (8.5 \pm 16.2) 90.6	0 (12.4 \pm 22.0) 123.5	0 (353.9 \pm 163.3) 390.2
Sierras y Llanuras de Durango	0 (9.7 \pm 14.7) 55	0 (6.4 \pm 11.1) 39.8	0 (4.7 \pm 9.4) 38.4	0 (6.1 \pm 11.8) 48.5	0 (11.7 \pm 18.4) 76.4	0.5 (43.0 \pm 41.4) 154.0	22.0 (106.4 \pm 45.7) 206.6	21.7 (120.9 \pm 67.9) 269.5	4.0 (111.7 \pm 88.7) 339.7	0 (25.1 \pm 30.9) 100.4	0 (9.4 \pm 13.3) 50.0	0.5 (10.2 \pm 16.1) 239	9.4 (350.2 \pm 15.7) 608.1
Gran Meseta y Cañones Chihuahuenses	0 (40.2 \pm 51.3) 189.4	0 (27.2 \pm 34.3) 130.9	0 (12.2 \pm 23.0) 112.0	0 (11.9 \pm 27.3) 143.0	0.0 (10.4 \pm 25.4) 131.2	0.1 (59.0 \pm 64.3) 280.3	6.7 (169.1 \pm 100.8) 459.9	36.6 (151.7 \pm 87.2) 405.6	2.5 (77.6 \pm 54.2) 234.5	0 (42.9 \pm 61.0) 210.5	0 (21.4 \pm 26.8) 103.7	0.7 (40.8 \pm 47.4) 184.8	10.1 (609.1 \pm 1416.4) 1502.1
Gran Meseta y Cañones Duranguenses	0.3 (52.5 \pm 52.1) 169.1	0 (31.8 \pm 35.5) 120.0	0 (23.6 \pm 41.7) 149.7	0 (17.6 \pm 28.0) 120.0	0.1 (19.6 \pm 26.8) 109.3	0 (105.9 \pm 73.4) 238.6	91.4 (255.4 \pm 104.7) 514.3	70.1 (229.5 \pm 90.6) 474.7	32 (152.1 \pm 67.9) 269.4	0 (64.5 \pm 56.9) 162.1	0 (30.3 \pm 38.1) 115.1	0.4 (64.1 \pm 63.2) 196.2	29.6 (922.3 \pm 34.4) 1502.1

6.5%); seven (nine; 4.8%); eight (nine; 4.8%); and nine (11; 5.9%). The most broadly distributed species, i.e., those inhabiting all nine physiographic regions, consist of one anuran (*Lithobates pustulosus**), nine squamates (*Phrynosoma cornutum*, *Sceloporus poinsettii*, *Urosaurus ornatus*, *Aspidoscelis exsanguis*, *Masticophis flagellum*, *Salvadora deserticola*, *Thamnophis cyrtopsis*, *T. eques*, and *Crotalus lepidus*), and one turtle (*Kinosternon hirtipes*). With one exception (the Mexican endemic *Lithobates pustulosus*), the remaining species also range northward in the USA.

Of the 186 species that comprise the herpetofauna of Chihuahua, 74 (or 39.8%) occupy one or two physiographic regions, which is of considerable conservation significance. The mean regional occupancy is 3.7. The number of species occupying a single physiographic region ranges from none in one of the nine regions (GMCD) to 28 in the GMCC region. The remaining six regions have single-digit numbers of single-region species, including the LMN (one), SPN (one), BDM (four), LSV (five), SCN (one), and SLD (two).

The physiographic region of greatest conservation importance is the Gran Meseta y Cañones Chihuahuenses, since it contains the largest number of species (128, including 27 anurans, three salamanders, 39 lizards, 53 snakes, and six turtles), the largest number of single-region species (28 species, including eight anurans, three lizards, 15 snakes, and two turtles), the largest number of country endemics (57), and the single state endemic (*Plestiodon multilineatus*).

The 28 single-region species that are restricted to the Gran Meseta y Cañones Chihuahuenses (* = endemic to Mexico, ** = endemic to Chihuahua, and *** = non-native to Chihuahua) are as follows:

- Agalychnis dacnicolor**
- Agkistrodon bilineatus*
- Crotalus basiliscus**
- Eleutherodactylus interorbitalis**
- Gastrophryne mazatlanensis*
- Gopherus evgoodei**
- Heloderma horridum**
- Holbrookia elegans*
- Hypopachus variolosus*
- Imantodes gemmistratus*
- Lampropeltis polyzona**
- Leptophis diplotropis**
- Lithobates cora**
- Lithobates lemosespinali**
- Micruroides euryxanthus*
- Micrurus distans**
- Oxybelis microphthalmus*
- Phyllodactylus saxatilis**
- Rena dugesii**
- Rhadinaea hesperia**
- Rhinella horribilis*
- Rhinoclemmys pulcherrima*
- Sympholis lippiens**
- Tantilla yaquia*
- Thamnophis validus**
- Tlalocohyla smithi**

Table 3. Composition of the native and non-native herpetofauna of Chihuahua, Mexico.

Order	Families	Genera	Species
Anura	8	14	35
Caudata	2	2	5
Subtotal	10	16	40
Squamata	19	59	133
Testudines	5	7	13
Subtotal	24	66	146
Total	34	82	186

*Trimorphodon tau**
*Tropidodipsas repleta**

Of these 28 single-region species, 18 (64.3%) are country endemics and 10 are non-endemics (35.7%).

The distribution of the following five species in Chihuahua is limited to the Llanuras y Sierras Volcánicas:

Agkistrodon laticinctus
Lampropeltis alterna
Nerodia erythrogaster
Pantherophis bairdi
Tantilla cucullata

All five of these species are non-endemics.

The following four species only occupy the Bolsón de Mapimí region in Chihuahua:

Ambystoma mavortium
*Indotyphlops braminus****
Rena segrega
*Uma paraphygas**

Two of these species are non-endemics, one is a country endemic, and one is a non-native.

The following two species are found only in the Sierras y Llanuras de Durango region in Chihuahua:

*Ambystoma velasci**
Salvadora lineata

One of these species is a country endemic and the other is a non-endemic.

Only a single species is restricted to each of the following three physiographic regions in Chihuahua. These three regions and the species involved are as follows:

Llanuras y Médanos del Norte—*Incilius alvarius*
Sierras Plegadas del Norte—*Eleutherodactylus marnockii*
Sierras y Cañadas del Norte—*Heloderma suspectum*

Each of these three species is a non-endemic.

Finally, no species are limited to the GMCD region in Chihuahua.

To determine the herpetofaunal relationships among the nine physiographic regions we recognize, we constructed a Coefficient of Biogeographic Resemblance (CBR) matrix (Table 6). As noted above, the number of species per physiographic region ranges from 58 in the SLD to 128 in the GMCC. The number of shared species

ranges from 14 between the SPN and GMCD regions to 72 between the SCN and GMCC regions. The range and mean of the shared species numbers for each of the nine regions, arranged according to the increasing mean number, are as follows (mean number in parentheses followed by range, then by total regional number in parentheses):

GMCD — (27.6) 14–59 (59)
SCN — (42.6) 29–72 (78)
SLD — (37.4) 22–42 (58)
BDM — (44.5) 20–62 (80)
SPN — (38.9) 14–58 (64)
LMN — (45.6) 23–62 (81)
SLT — (40.1) 27–53 (61)
GMCC — (46.1) 27–72 (128)
LSV — (41.6) 17–61 (75)

Notably, with one exception, as the total regional number increases, so does the mean number of shared species.

The CBR data in Table 6 demonstrate a range of values from 0.23 to 0.83. The lowest value is that between the SPN and the GMCD, while the highest value is that between the SPN and the LSV. The highest CBR value for each of the nine regions is as follows:

LMN (81) — 0.77 — BDM (80)
SLT (61) — 0.76 — SCN (78)
SPN (64) — 0.83 — LSV (75)
GMCC (128) — 0.70 — SCN (78)
BDM (80) — 0.81 — SPN (64)
SLD (58) — 0.66 — SLT (61)
LSV (75) — 0.83 — SPN (64)
GMCD (59) — 0.63 — GMCC (128)
SCN (78) — 0.76 — SLT (61)

For the nine regions, the strongest relationships involve only six of the other regions (SPN, BDM, LSV, SCN, SLT, and GMCC). The SPN, SCN, and SLT are involved in two of these relationships, whereas the other three (BDM, LSV, and GMCC) are involved only once.

Based on the data in Table 6, we constructed a UPGMA dendrogram to conveniently illustrate the overall herpetofaunal resemblances among the nine physiographic regions in Chihuahua in a hierarchical fashion (Fig. 13). The dendrogram indicates that the nine physiographic regions are divided into two principal groups separated at the 0.40 level, one containing the GMCC and GMCD regions and the other containing the remaining seven regions. The GMCC and GMCD regions are located adjacent to one another in southwestern Chihuahua (Fig. 1), with the smaller GMCD region situated to the south of the GMCC region. The other seven regions are partitioned into two groups separated at the 0.53 level. One group comprises the SCN, SLT, and SLD regions, which are located in a swath lying to the east of the GMCC–GMCD pair that extends from the west-central portion of the state adjacent to the border of Sonora southeastward to the south-central portion adjacent to the border of Durango (Fig. 1). The SCN and SLT regions are separated at the 0.76 level and lie

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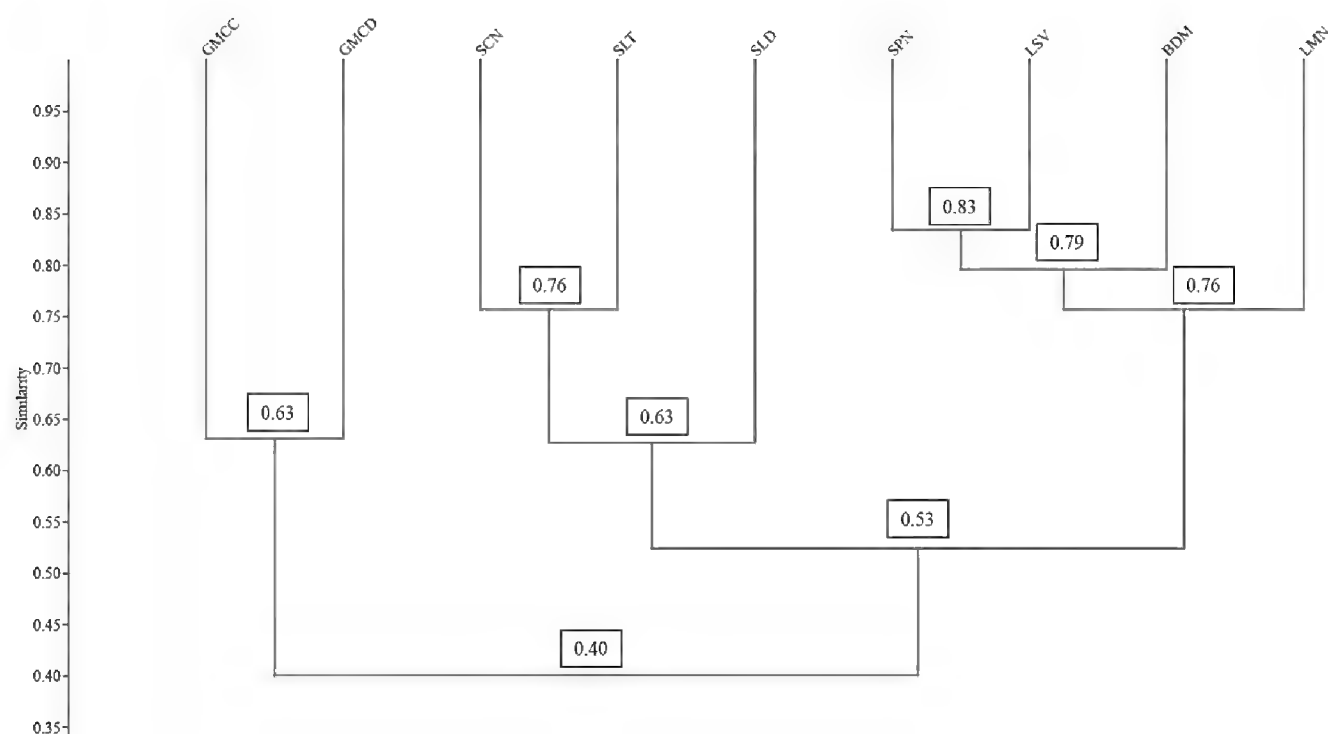


Fig. 13. UPGMA-generated dendrogram showing the similarity relationships of species richness among the herpetofaunal components of the nine physiographic provinces of Chihuahua (based on the data in Table 6; Sokal and Michener 1958). The similarity values were calculated using the Coefficient of Biogeographic Resemblance (CBR) of Duellman (1990).

adjacent to one another, with the SCN and SLT regions separated from the SLD region at the 0.63 level. Finally, the remaining four regions (LMN, SDM, LSV, and SPN) are joined to one another at the 0.76 level; these four regions are situated in a group occupying roughly the eastern half of the state (Fig. 1) and are comprised of two swaths (LMN–BDM and SPN–LSV), the latter lying to the east of the former. The two most closely related regions (at the 0.83 level) are the SPN and LSV regions that lie adjacent to one another along the borders of Texas and Coahuila. These two regions are connected to the BDM region, which is adjacent to both these regions, at the 0.79 level. Finally, as noted above, these three regions are connected to the LMN region, located in the northwestern section of the state adjacent to the border of New Mexico, at the 0.76 level.

Distribution Status Categorizations

To categorize the distributional status of members of the Chihuahua herpetofauna, we used the system in Alvarado-Díaz et al. (2013) and the other MCS entries (see above). We indicate our assignment of the four distributional categories of non-endemic, country endemic, state endemic, and non-native species in Table 7 and summarize the data in Table 8.

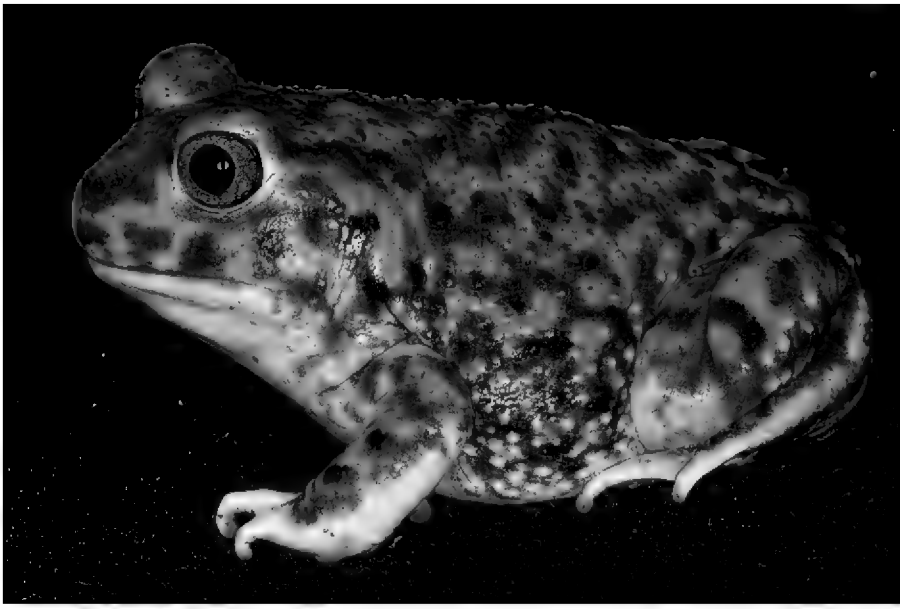
The number and proportion of species in each of these four categories are as follows: non-endemic, 121 of 186 (65.1%); country endemics, 61 (32.8%); non-natives, three (1.6%); and state endemics, one (0.5%). Accordingly, the Chihuahua herpetofauna resembles the other faunas dealt with in the MCS that have more non-native species than in any other category, and in this case, all the other categories combined (121 vs. 65). The number of non-endemic species exceeds that of the country endemic species in Oaxaca (Mata-Silva et al. 2015, 2021), Chiapas (Johnson et al. 2015a), Nuevo León (Nevárez-de los Reyes et al. 2016), the Mexican Yucatan Peninsula (González-Sánchez et al. 2017), Coahuila (Lazcano et al. 2019), Veracruz (Torres-Hernández et al. 2021), Tabasco (Barragán-

Vázquez et al. 2022), and the Baja California Peninsula (Peralta-García et al. 2023). In the other states dealt with thus far in the MCS, the number of country endemics is greater than that of the non-endemic species in Michoacán (Alvarado-Díaz et al. 2013), Nayarit (Woolrich-Piña et al. 2016), Jalisco (Cruz Sáenz et al. 2017), Puebla (Woolrich-Piña et al. 2017), Hidalgo (Ramírez-Bautista et al. 2020), Querétaro (Cruz-Elizalde et al. 2022), and Guanajuato (Leyte-Manrique et al. 2022).

As indicated above, in some entries of the MCS the number of country endemics is greater than the number of non-endemic species, whereas in others the reverse is the case. Thus, the ratios of country endemics to non-endemic species vary considerably, from 0.53 in Jalisco to 0.95 in Baja California. The ratios in which the number of non-endemics exceeds that of the country endemics range from 1.12 in Oaxaca to 127.0 in the Mexican Yucatan Peninsula. The figure for Chihuahua is 1.9 (121/65). As noted by Leyte-Manrique et al. (2022: 147), “the nature of this ratio depends on how close the state in question is to either the United States or Central America. This ratio also depends upon the size of these aspects of a given herpetofauna as to whether the ratio will be more or less than one.” In the case of Chihuahua, we would expect the ratio to be greater than one since this state shares a relatively long border with the USA. Leyte-Manrique et al. (2022) quoted Torres-Hernández et al. (2021), who stated: “In the case of the three MCS states that border the USA, the ratios are 3.22 (100/31 in Coahuila; Lazcano et al. 2019), 2.44 (95/39 in Nuevo León; Nevárez-de los Reyes et al. 2016), and 2.32 (130/56 in Tamaulipas; Terán-Juárez et al. 2016).” As noted above, the ratio for Chihuahua is 1.9 (121/65), which is most similar to the ratio for Tamaulipas (i.e., 119/49=2.4; Terán-Juárez et al. 2016).

Interestingly, only one state endemic species is known to occur in Chihuahua, i.e., *Plestiodon multilineatus* (McCranie and Wilson 1987). This skink inhabits pine forests at elevations from 2,246 to 2,615 m in the Sierra Madre Occidental (Tanner 1988).

The three non-native species recorded from Chihuahua (Table 7) are the anuran *Lithobates catesbeianus*, the gecko



No. 13. *Scaphiopus couchii* Baird, 1854. The distribution of Couch's Spadefoot "extends from southeastern California to southeastern Colorado and southwestern Oklahoma, in the United States, southward in Mexico to northern Nayarit, Zacatecas, San Luis Potosí, and northern Veracruz" (Lemos-Espinal and Dixon 2013: 75–76). This individual was photographed in scrub vegetation near the city of Chihuahua, Chihuahua. Wilson et al. (2013b) established its EVS as 3, placing it at the lower limit of the low vulnerability category. Its IUCN status has been calculated as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 14. *Spea bombifrons* (Cope, 1863). The Plains Spadefoot is distributed from "southern Alberta and southwestern Saskatchewan (Canada) southward through Montana, North Dakota, ... Nebraska and eastern Colorado to southeastern Utah, northeastern and southeastern Arizona, central Missouri, Oklahoma and western and South Texas (USA) to Chihuahua and Tamaulipas (Mexico)" (Frost 2023). This individual was photographed in sand dune vegetation containing an aromatic shrub (*Poliomintha incana*) and Sand Sagebrush (*Artemisia filifolia*) at Rancho Zorro Plateado in the city of Juárez, Chihuahua. Wilson et al. (2013b) calculated its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN rated this species as Least Concern, and this species is not listed by SEMARNAT. *Photo by Jesús M. Martínez-Calderas.*



No. 15. *Spea multiplicata* (Cope, 1863). The distribution of the Mexican Spadefoot "extends from southeastern Utah and southern Colorado through western Oklahoma, Arizona and New Mexico, in the United States, southward into Mexico to northern Sonora and throughout the Chihuahuan Desert to the Transvolcanic Belt; it also occurs in mountainous regions from the Sierra Madre Occidental of central Chihuahua southward to Oaxaca" (Lemos-Espinal and Dixon 2013: 76–77). This individual was photographed in a sand dune environment (medanos) near Rancho El Lobo, in the city of Juárez, Chihuahua. Wilson et al. (2013b) determined its EVS as 6, placing it in the middle of the low vulnerability category. The IUCN has established its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 16. *Ambystoma rosaceum* Taylor, 1941. The Tarahumara Salamander occurs at "high elevations in the Sierra Madre Occidental from the region of Aguascalientes and western Zacatecas north through montane Nayarit, Durango and montane Sinaloa to northern Chihuahua (vicinity of Casas Grandes) and northern Sonora (Sierra Pinitos, Sierra de los Ajos, Sierra El Tigre, and Sierra San Luis in the north), Mexico" (Frost 2023). This larval individual was photographed in pine forest vegetation at Monterde, in the municipality of Guazapares, Chihuahua. Wilson et al. (2013b) ascertained its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN conservation status of this salamander has been assessed as Least Concern, and this species was placed in the Special Protection category by SEMARNAT. *Photo by Eric Centenero-Alcalá.*

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Table 4. Distribution of the amphibians, squamates, and turtles of Chihuahua, Mexico, by physiographic region (= sub physiographic provinces). Abbreviations: Sierra y Llanuras del Norte - Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Bolsón de Mapimí (BDM), Llanuras y Sierras Volcánicas (LSV); Sierra Madre Occidental - Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Gran Meseta y Cañones Chihuahuenses (GMCC), Sierras y Llanuras de Durango (SLD), and Gran Meseta y Cañones Duranguenses (GMCD). See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species.

Taxon	Physiographic region									Number of regions occupied
	Sierra y Llanuras del Norte				Sierra Madre Occidental					
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Amphibia (40 species)										
Anura (35 species)										
Bufonidae (11 species)										
Anaxyrus cognatus	+	+	+	+	+	+		+		7
Anaxyrus debilis	+	+	+	+						4
Anaxyrus mexicanus *	+				+	+	+	+	+	6
Anaxyrus punctatus	+	+	+	+	+	+	+	+		8
Anaxyrus speciosus	+		+	+	+		+			5
Anaxyrus woodhousii	+	+	+	+	+	+	+	+		8
Incilius alvarius	+									1
Incilius mazatlanensis *							+		+	2
Incilius mccoysi *					+		+		+	3
Incilius occidentalis *					+		+			2
Rhinella horribilis							+			1
Craugastoridae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Craugastor augusti					+	+	+			3
Craugastor tarahumaraensis *					+		+	+		3
Eleutherodactylidae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Eleutherodactylus interorbitalis *							+			1
Eleutherodactylus marnockii		+								1
Hylidae (4 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Dryophytes arenicolor	+			+	+	+	+	+	+	7
Dryophytes wrightorum					+	+	+			3
Smilisca baudinii					+		+			2
Tlalocohyla smithii *							+			1
Microhylidae (3 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Gastrophryne mazatlanensis							+			1
Gastrophryne olivacea	+	+	+	+						4
Hypopachus variolosus							+			1
Phyllomedusidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Agalychnis dacnicolor *							+			1
Ranidae (9 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Lithobates berlandieri	+	+	+	+			+	+	+	7
Lithobates catesbeianus ***	+			+						2
Lithobates chiricahuensis	+		+		+	+	+	+		6
Lithobates cora*							+			1
Lithobates lemosespinali *							+			1
Lithobates magnaocularis *					+	+	+			3
Lithobates pustulosus *	+	+	+	+	+	+	+	+	+	9
Lithobates tarahumarae					+		+		+	3
Lithobates yavapaiensis					+		+			2
Scaphiopodidae (3 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Scaphiopus couchii	+	+	+	+				+		5
Spea bombifrons	+		+	+						3
Spea multiplicata	+	+	+	+	+	+	+	+		8
Caudata (5 species)										
Ambystomatidae (4 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Ambystoma mavortium			+							1
Ambystoma rosaceum *			+		+	+	+		+	5
Ambystoma silvense *					+	+	+	+		4
Ambystoma velasci *								+		1

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Chihuahua, Mexico, by physiographic region (= sub physiographic provinces). Abbreviations: Sierra y Llanuras del Norte - Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Bolsón de Mapimí (BDM), Llanuras y Sierras Volcánicas (LSV); Sierra Madre Occidental - Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Gran Meseta y Cañones Chihuahuenses (GMCC), Sierras y Llanuras de Durango (SLD), and Gran Meseta y Cañones Duranguenses (GMCD). See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species.

Taxon	Physiographic region									Number of regions occupied
	Sierra y Llanuras del Norte				Sierra Madre Occidental					
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
Reptilia (146 species)										
Squamata (133 species)										
Squamata/Lacertilia (53 species)										
Anguidae (5 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Barisia ciliaris</i> *							+	+		2
<i>Barisia imbricata</i> *					+	+	+			3
<i>Barisia levicollis</i> *					+	+	+			3
<i>Elgaria kingii</i>			+		+	+	+		+	5
<i>Gerrhonotus infernalis</i>	+			+	+		+	+		5
Anolidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Norops nebulosus</i> *							+		+	2
Crotaphytidae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Crotaphytus collaris</i>	+	+	+	+						4
<i>Gambelia wislizenii</i>	+	+	+	+						4
Eublepharidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Coleonyx brevis</i>		+	+	+						3
Gekkonidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Hemidactylus turcicus</i> ***		+	+	+						3
Helodermatidae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Heloderma horridum</i> *							+			1
<i>Heloderma suspectum</i>					+					1
Iguanidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Ctenosaura macrolopha</i> *							+		+	2
Phrynosomatidae (23 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Cophosaurus texanus</i>	+	+	+	+				+		5
<i>Holbrookia approximans</i> *	+	+	+	+	+	+	+	+		8
<i>Holbrookia elegans</i>							+			1
<i>Holbrookia maculata</i>	+	+	+	+	+	+	+	+		8
<i>Phrynosoma cornutum</i>	+	+	+	+	+	+	+	+	+	9
<i>Phrynosoma hernandesi</i>	+		+		+	+	+	+		6
<i>Phrynosoma modestum</i>	+	+	+	+		+				5
<i>Phrynosoma orbiculare</i> *					+	+	+		+	4
<i>Sceloporus albiventris</i> *							+	+	+	3
<i>Sceloporus clarkii</i>	+				+		+	+	+	5
<i>Sceloporus cowlesi</i>	+	+	+	+						4
<i>Sceloporus jarrovi</i>	+		+		+	+	+	+	+	7
<i>Sceloporus lemosespinali</i> *					+	+	+		+	4
<i>Sceloporus magister</i>	+	+	+	+						4
<i>Sceloporus merriami</i>		+		+						2
<i>Sceloporus nelsoni</i> *							+		+	2
<i>Sceloporus poinsettii</i>	+	+	+	+	+	+	+	+	+	9
<i>Sceloporus slevini</i>			+		+	+	+	+	+	6
<i>Sceloporus virgatus</i>	+				+	+	+			4
<i>Uma paraphygas</i> *			+							1
<i>Urosaurus bicarinatus</i> *							+	+	+	3
<i>Urosaurus ornatus</i>	+	+	+	+	+	+	+	+	+	9
<i>Uta stansburiana</i>	+	+	+	+	+	+	+	+		8
Phyllodactylidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Phyllodactylus saxatilis</i> *							+			1
Scincidae (7 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Plestiodon bilineatus</i> *		+			+		+	+		4

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Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Chihuahua, Mexico, by physiographic region (= sub physiographic provinces). Abbreviations: Sierra y Llanuras del Norte - Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Bolsón de Mapimí (BDM), Llanuras y Sierras Volcánicas (LSV); Sierra Madre Occidental - Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Gran Meseta y Cañones Chihuahuenses (GMCC), Sierras y Llanuras de Durango (SLD), and Gran Meseta y Cañones Duranguenses (GMCD). See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species.

Taxon	Physiographic region									Number of regions occupied
	Sierra y Llanuras del Norte				Sierra Madre Occidental					
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Plestiodon multivirgatus</i>	+				+		+		+	4
<i>Plestiodon obsoletus</i>	+	+	+	+		+		+		6
<i>Plestiodon parviauriculatus</i> *					+		+		+	3
<i>Plestiodon tetragrammus</i>		+	+	+	+	+	+		+	7
Teiidae (9 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Aspidoscelis costatus</i> *							+		+	2
<i>Aspidoscelis exsanguis</i>	+	+	+	+	+	+	+	+	+	9
<i>Aspidoscelis gularis</i>		+	+	+	+	+	+	+		7
<i>Aspidoscelis inornata</i>	+	+	+	+		+	+		+	7
<i>Aspidoscelis marmorata</i>	+	+	+	+		+	+			6
<i>Aspidoscelis neomexicanus</i>	+		+							2
<i>Aspidoscelis sonorae</i>	+				+					2
<i>Aspidoscelis tessellata</i>	+	+	+	+		+	+	+		7
<i>Aspidoscelis uniparens</i>	+	+	+	+	+	+	+	+		8
Squamata/Serpentes (80 species)										
Boidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Boa sigma</i> *							+		+	2
Colubridae (40 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Arizona elegans</i>	+	+	+	+				+		5
<i>Bogertophis subocularis</i>		+	+	+						3
<i>Conopsis nasus</i> *					+		+		+	3
<i>Drymarchon melanurus</i>							+		+	2
<i>Drymobius margaritiferus</i>							+		+	2
<i>Gyalopion canum</i>	+		+							2
<i>Gyalopion quadrangulare</i>							+		+	2
<i>Lampropeltis alterna</i>				+						1
<i>Lampropeltis knoblochi</i>					+	+	+	+		4
<i>Lampropeltis polyzona</i> *							+			1
<i>Lampropeltis splendida</i>	+	+		+				+		4
<i>Leptophis diplotropis</i> *							+			1
<i>Masticophis bilineatus</i>	+				+		+		+	4
<i>Masticophis flagellum</i>	+	+	+	+	+	+	+	+	+	9
<i>Masticophis mentovarius</i>				+			+		+	3
<i>Masticophis taeniatus</i>	+	+	+	+	+	+	+	+		8
<i>Mastigodryas cliftoni</i> *							+		+	2
<i>Opheodrys vernalis</i>					+		+		+	3
<i>Oxybelis microphthalmus</i>							+			1
<i>Pantherophis bairdi</i>				+						1
<i>Pantherophis emoryi</i>	+	+	+	+				+		5
<i>Pituophis catenifer</i>	+	+	+	+	+	+	+	+		8
<i>Pituophis deppei</i> *			+				+			2
<i>Rhinocheilus lecontei</i>	+	+	+	+						4
<i>Salvadora bairdi</i> *					+		+			2
<i>Salvadora deserticola</i>	+	+	+	+	+	+	+	+	+	9
<i>Salvadora grahamiae</i>	+		+	+	+	+		+		6
<i>Salvadora lineata</i>								+		1
<i>Senticolis triaspis</i>	+				+		+		+	4
<i>Sonora aemula</i> *							+		+	2
<i>Sonora semiannulata</i>	+	+	+	+		+				5
<i>Sympholis lippiens</i> *							+			1
<i>Tantilla atriceps</i>		+	+	+						3

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Chihuahua, Mexico, by physiographic region (= sub physiographic provinces). Abbreviations: Sierra y Llanuras del Norte - Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Bolsón de Mapimí (BDM), Llanuras y Sierras Volcánicas (LSV); Sierra Madre Occidental - Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Gran Meseta y Cañones Chihuahuenses (GMCC), Sierras y Llanuras de Durango (SLD), and Gran Meseta y Cañones Duranguenses (GMCD). See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species.

Taxon	Physiographic region									Number of regions occupied
	Sierra y Llanuras del Norte				Sierra Madre Occidental					
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Tantilla nigriceps</i>	+	+	+				+			4
<i>Tantilla wilcoxi</i>					+	+	+	+		4
<i>Tantilla yaquia</i>							+			1
<i>Trimorphodon tau</i> *							+			1
<i>Trimorphodon wilkinsonii</i>	+	+	+							3
Dipsadidae (10 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Diadophis punctatus</i>	+			+	+		+		+	5
<i>Geophis dugesii</i> *							+		+	2
<i>Heterodon kennerlyi</i>	+	+	+	+	+	+				6
<i>Hypsiglena chlorophaea</i>	+	+	+	+		+		+		6
<i>Hypsiglena jani</i>		+	+	+				+		4
<i>Imantodes gemmistratus</i>							+			1
<i>Leptodeira splendida</i> *							+		+	2
<i>Rhadinaea hesperia</i> *							+			1
<i>Rhadinaea laureata</i> *							+		+	2
<i>Tropidodipsas repleta</i> *							+			1
Elapidae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Micruroides euryxanthus</i>							+			1
<i>Micrurus distans</i> *							+			1
Leptotyphlopidae (4 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Rena dugesii</i> *							+			1
<i>Rena dulcis</i>	+			+				+		3
<i>Rena humilis</i>	+		+	+			+			4
<i>Rena segregata</i>			+							1
Natricidae (11 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Nerodia erythrogaster</i>				+						1
<i>Storeria storerioides</i> *					+		+		+	3
<i>Thamnophis cyrtopsis</i>	+	+	+	+	+	+	+	+	+	9
<i>Thamnophis elegans</i>			+		+	+	+		+	5
<i>Thamnophis eques</i>	+	+	+	+	+	+	+	+	+	9
<i>Thamnophis errans</i> *					+	+	+	+	+	5
<i>Thamnophis marciatus</i>	+	+	+	+		+		+		6
<i>Thamnophis melanogaster</i> *					+		+			2
<i>Thamnophis sirtalis</i>	+		+		+	+	+		+	6
<i>Thamnophis unilabialis</i> *					+		+	+	+	4
<i>Thamnophis validus</i> *							+			1
Typhlopidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Indotyphlops braminus</i> ***			+							1
Viperidae (11 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Agkistrodon bilineatus</i>							+			1
<i>Agkistrodon laticinctus</i>				+						1
<i>Crotalus atrox</i>	+	+	+	+	+			+		6
<i>Crotalus basiliscus</i> *							+			1
<i>Crotalus lepidus</i>	+	+	+	+	+	+	+	+	+	9
<i>Crotalus molossus</i>					+	+	+	+	+	5
<i>Crotalus ornatus</i>	+	+	+	+						4
<i>Crotalus pricei</i>					+	+	+		+	4
<i>Crotalus scutulatus</i>	+	+	+	+	+	+		+		7
<i>Crotalus viridis</i>	+	+								2
<i>Crotalus willardi</i>					+	+	+		+	4
Testudines (13 species)										

The herpetofauna of Chihuahua, Mexico

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Chihuahua, Mexico, by physiographic region (= sub physiographic provinces). Abbreviations: Sierra y Llanuras del Norte - Llanuras y Médanos del Norte (LMN), Sierras Plegadas del Norte (SPN), Bolsón de Mapimí (BDM), Llanuras y Sierras Volcánicas (LSV); Sierra Madre Occidental - Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), Gran Meseta y Cañones Chihuahuenses (GMCC), Sierras y Llanuras de Durango (SLD), and Gran Meseta y Cañones Duranguenses (GMCD). See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species.

Taxon	Physiographic region									Number of regions occupied
	Sierra y Llanuras del Norte				Sierra Madre Occidental					
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Terrapene nelsoni</i> *	+						+		+	3
<i>Terrapene ornata</i>	+	+	+	+						4
<i>Trachemys gaigeae</i>	+	+	+							3
Geoemydidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Rhinoclemmys pulcherrima</i>							+			1
Kinosternidae (5 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Kinosternon durangoense</i> *			+	+						2
<i>Kinosternon flavescens</i>	+	+		+						3
<i>Kinosternon hirtipes</i>	+	+	+	+	+	+	+	+	+	9
<i>Kinosternon integrum</i> *	+						+			2
<i>Kinosternon sonoriense</i>	+				+	+	+			4
Testudinidae (2 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Gopherus evgoodei</i> *							+			1
<i>Gopherus flavomarginatus</i> *			+	+						2
Trionychidae (1 species)	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD	
<i>Apalone spinifera</i>	+	+	+	+						4
Total (186 species)										

Hemidactylus turcicus, and the blindsnake *Indotyphlops braminus*. The blindsnake *I. braminus* is one of the most widespread non-native species in Mexico, as it has been recorded in all 16 previous MCS entries. González-Sánchez et al. (2021) also indicated this snake is one of the most widespread of the introduced herpetofaunal taxa in Mesoamerica (Mexico and Central America), as it has been recorded in six of the eight countries in this region, and this blindsnake also occurs in northwestern Costa Rica (Wallach 2020a; Solórzano 2022).

Wilson et al. (2017) developed a system for categorizing the distribution of the non-endemic species of the Mesoamerican herpetofauna, including those of Mexico. As expected, the categorization of the non-endemic species in Chihuahua (Table 9) demonstrates that most of them (108 of 121 or 89.3%) are placed in the MXUS category. This situation was expected given that Chihuahua is one of the six Mexican states along the Mexico–USA border, as well as the one with the longest border. The next most common categorization is USCA (six, or 5.0%), which also was expected since Chihuahua lies at the opposite end of the country of Mexico from any of the countries in Central America. The MXSA and MXCA species each amount to three (2.5%). Finally, a single USSA species is represented (0.8%). Thus, all but six of the non-endemic species have distributional ranges that extend northward into the USA, and in a few cases all the way to South America.

Herpetofaunal Comparisons in Adjacent Mexican States

As indicated above, the northern border of Chihuahua is part of the border between Mexico and the USA. The rest of Chihuahua’s border is contiguous with parts of

the Mexican states of Sonora, Sinaloa, Durango, and Coahuila. The herpetofauna of Coahuila was examined in the Mexican Conservation Series (Lazcano et al. 2019), and the herpetofauna of Durango, Sinaloa, and Sonora were dealt with by Lemos-Espinal et al. (2018a, 2019b, 2020).

In Table 10 we compare the herpetofauna of Chihuahua to those of the five bordering Mexican states, which indicates the total number of herpetofaunal species, the number of endemic species (both state and country, as applicable), the number of non-native species, and the percentage of endemism (calculated as the number of endemic species [state and country endemics] divided by the number of native species times 100).

The size of the herpetofauna in each of these five states ranges from 143 in Coahuila to 200 in Sonora (Table 10). The number of non-endemic species ranges from 73 in Sinaloa to 125 in Sonora. The number of endemic species ranges from 40 in Coahuila to 82 in Sinaloa. In most of these five states the number of non-endemic species is higher than the number of endemic species, except for Sinaloa. The ratios of endemic species to non-endemic species for the five states are as follows: Sonora (68/125 = 0.54); Chihuahua (62/121 = 0.51); Coahuila (40/100 = 0.40); Durango (72/81 = 0.89); and Sinaloa (82/73 = 1.12). Evidently, the lower ratios are those for Coahuila, Chihuahua, and Sonora, all states that share a border with the USA; the higher ratios are those for Durango and Sinaloa, states that do not border the USA. The percentage of endemism in the five states ranges from 28.6 in Coahuila to 52.9 in Sinaloa, with a mean value of 39.4. Finally, the number of non-native species ranges from three to seven. Three of the five states harbor three non-native species (Chihuahua,

Table 5. Distributional summary of the herpetofaunal families in Chihuahua, Mexico, by physiographic region. See Table 4 for an explanation of the abbreviations.

Family	Number of species	Distributional occurrence								
		LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD
Bufonidae	11	7	4	5	5	7	4	8	4	3
Craugastoridae	2	—	—	—	—	2	1	2	1	—
Eleutherodactylidae	2	—	1	—	—	—	—	1	—	—
Hylidae	4	1	—	—	1	3	2	4	1	1
Microhylidae	3	1	1	1	1	—	—	2	—	—
Phyllomedusidae	1	—	—	—	—	—	—	1	—	—
Ranidae	9	4	2	3	3	5	3	8	3	3
Scaphiopodidae	3	3	2	3	3	1	1	1	2	—
Subtotal	35	16	10	12	13	18	11	27	11	7
Ambystomatidae	4	—	—	2	—	2	2	2	2	1
Plethodontidae	1	—	—	—	—	1	—	1	—	—
Subtotal	5	—	—	2	—	3	2	3	2	1
Amphibians total	40	16	10	14	13	21	13	30	13	8
Anguidae	5	1	—	1	1	4	3	5	2	1
Anolidae	1	—	—	—	—	—	—	1	—	1
Crotaphytidae	2	2	2	2	2	—	—	—	—	—
Eublepharidae	1	—	1	1	1	—	—	—	—	—
Gekkonidae	1	—	1	1	1	—	—	—	—	—
Helodermatidae	2	—	—	—	—	1	—	1	—	—
Iguanidae	1	—	—	—	—	—	—	1	—	1
Phrynosomatidae	23	14	11	14	11	13	13	17	13	11
Phyllodactylidae	1	—	—	—	—	—	—	1	—	—
Scincidae	7	2	3	2	2	6	3	6	2	4
Teiidae	9	7	6	7	6	4	6	7	4	3
Subtotal	53	26	24	28	24	28	25	39	21	21
Boidae	1	—	—	—	—	—	—	1	—	1
Colubridae	40	16	14	16	17	12	8	26	11	12
Dipsadidae	10	3	3	3	4	2	2	7	2	4
Elapidae	2	—	—	—	—	—	—	2	—	—
Leptotyphlopidae	4	2	—	2	2	—	—	2	1	—
Natricidae	11	4	3	5	4	8	6	9	5	7
Typhlopidae	1	—	—	1	—	—	—	—	—	—
Viperidae	11	5	5	4	5	6	5	6	4	4
Subtotal	80	30	25	31	32	28	21	53	23	28
Emydidae	4	4	2	3	1	—	—	1	—	1
Geoemydidae	1	—	—	—	—	—	—	1	—	—
Kinosternidae	5	4	2	2	3	2	2	3	1	1
Testudinidae	2	—	—	1	1	—	—	1	—	—
Trionychidae	1	1	1	1	1	—	—	—	—	—
Subtotal	13	9	5	7	6	2	2	6	1	2
Reptiles total	146	65	54	66	62	58	48	98	45	51
Sum total	186	81	64	80	75	78	61	128	58	59



No. 17. *Barisia ciliaris* (Smith, 1942). The distribution of the Sierra Alligator Lizard “extends along the Sierra Madre Oriental, from Nuevo León and southeastern Coahuila southward to at least Guanajuato, and northward along the Sierra Madre Occidental to extreme southern Chihuahua” (Lemos-Espinal and Dixon 2013: 96–97). This individual was photographed in pine-oak forest at 25 km E of Guachochi on highway 23, in the municipality of Balleza, Chihuahua. Wilson et al. (2013a) determined its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN has not evaluated its conservation status, and this species is not listed by SEMARNAT. *Photo by Antonio Esau Valdenegro Brito.*



No. 18. *Barisia levicollis* Stejneger, 1890. The Chihuahuan Alligator Lizard is distributed in Chihuahua (Lemos-Espinal and Smith 2007), and it also might occur in eastern Sonora (Rorabaugh and Lemos-Espinal 2016). This individual was photographed in a remnant patch of pine forest at La Mesa de Cristo Rey, in the municipality of Guerrero, Chihuahua. Wilson et al. (2013a) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN has evaluated its conservation status as Data Deficient, and it was noted as a species of Special Protection by SEMARNAT. *Photo by José Eduardo Gámez López.*



No. 19. *Elgaria kingii* Gray, 1838. The Madrean Alligator Lizard “lives in and adjacent to mountains of eastern Arizona, southwestern New Mexico, and in the Sierra Madre in eastern Sonora and western Chihuahua ... to Jalisco” (Lemos-Espinal and Smith 2007). This individual was photographed in pine forest at Divisadero, in the municipality of Urique, Chihuahua. Wilson et al. (2013a) determined the EVS of this anguid as 10, placing it at the lower limit of the medium vulnerability category. The IUCN has judged its conservation status as Least Concern, and it was placed in the Special Protection category by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 20. *Gerrhonotus infernalis* Baird, 1859. The Texas Alligator Lizard occurs from “central Texas west to the Big Bend area, and west of the Sierra Madre Oriental to southern San Luis Potosí and perhaps extreme southeastern Durango. Its distribution in Mexico is limited to the Chihuahuan Desert and Sierra Madre Oriental biotic provinces...” (Lemos-Espinal et al. 2018). This adult individual was photographed in pine-oak forest at Sierra Rica, in the municipality of Manuel Benavides, Chihuahua. Wilson et al. (2013a) assessed its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by José Candelario Hernández Álvarez.*

Table 6. Pair-wise comparison matrix of the Coefficient of Biogeographic Resemblance (CBR) data of herpetofaunal relationships for the nine physiographic regions in Chihuahua, Mexico. Underlined values = number of species in each region; upper triangular matrix values = species in common between two regions; and lower triangular matrix values = CBR values. The formula for this algorithm is $CBR = 2C/N_1 + N_2$ (Duellman 1990), where C is the number of species in common to both regions, N_1 is the number of species in the first region, and N_2 is the number of species in the second region. See Table 4 for abbreviations. See Fig. 11 for the UPGMA dendrogram produced from the CBR data.

	Physiographic region								
	LMN	SPN	BDM	LSV	SCN	SLT	GMCC	SLD	GMCD
LMN	<u>81</u>	54	62	59	41	40	44	42	23
SPN	0.74	<u>64</u>	58	58	29	33	29	36	14
BDM	0.77	0.81	<u>80</u>	61	36	42	38	39	20
LSV	0.76	0.83	0.79	<u>75</u>	31	35	33	39	17
SCN	0.52	0.41	0.46	0.41	<u>78</u>	53	72	40	39
SLT	0.56	0.53	0.60	0.51	0.76	<u>61</u>	52	39	27
GMCC	0.42	0.30	0.37	0.33	0.70	0.55	<u>128</u>	42	59
SLD	0.60	0.59	0.57	0.59	0.59	0.66	0.45	<u>58</u>	22
GMCD	0.33	0.23	0.29	0.25	0.57	0.45	0.63	0.38	<u>59</u>

Coahuila, and Durango), one supports four species (Sinaloa), and one contains seven species (Sonora). The total number of non-native species in these five states is nine, which includes two anurans (*Lithobates catesbeianus* in Chihuahua, Coahuila, Durango, Sinaloa, and Sonora, and *L. berlandieri* in Sonora), four lizards (*Gehyra mutilata* in Sinaloa, *Hemidactylus frenatus* in Sinaloa and Sonora, *H. turcicus* in Chihuahua, Coahuila, Durango, and Sonora, and *Sauromalus hispidus* in Sonora), one snake (*Indotyphlops braminus* in Chihuahua, Durango, Sinaloa, and Sonora), and two turtles (*Trachemys scripta* in Coahuila and *Apalone spinifera* in Sonora). The most widespread of these nine non-native species are *Lithobates catesbeianus* (five states), and *Hemidactylus turcicus* and *Indotyphlops braminus* (four states each).

Principal Environmental Threats

In each entry of the Mexican Conservation Series (see description above) we concluded that the inclusion of species within the state’s system of protected areas is an important step toward assuring the safety of the herpetofauna from anthropogenic threats. In doing so, several assumptions were made. A major assumption is that the system of natural protected areas (NPAs) is so extensive and well protected that the perpetuity of the herpetofauna is guaranteed. This assumption is based on the premise that the integrity of the NPAs is protected from direct impacts by human action for the long term (e.g., land conversion), and also that these areas will somehow remain unimpacted by over-arching global environmental threats such as climate change and water pollution. Thus, even given that the maintenance of sufficiently inclusive NPAs is an important first step in the direction of long-term support for the herpetofaunal populations, broad-scale studies of the impact of humanity on the nine “planetary boundaries” indicate that our species has exceeded the “safe operating space” within the last few years (Richardson et al. 2023).

A summary piece based this paper indicated that: “back in 2009 researchers published the first report on exceeding the boundaries of Earth’s ‘safe operating space.’ In 2015, there was an update, which concluded that the Earth had crossed the line beyond safety on at least four of nine boundaries (i.e., climate change, land use, biodiversity, and nutrient

flows). Now, the researchers have provided an even more disturbing update assessing the planet’s health (Earth beyond six of nine planetary boundaries), in which fresh water and forests were added to the list. Only three boundaries, stratospheric ozone levels, air pollution, and ocean acidification are still inside the safety zone, and the two latter areas are edging up to the boundary. The researchers noted: Currently, anthropogenic perturbations of the global environment are primarily addressed as if they were separate issues, e.g., climate change, biodiversity loss, or pollution. This approach, however, ignores these perturbations’ nonlinear interaction and resulting aggregate effects on the overall state of Earth’s system. Planetary boundaries bring a scientific understanding of anthropogenic global environmental impacts into a framework that calls for considering the state of Earth’s system as a whole.”

Whereas this last conclusion appears “earth-shaking” (no pun intended), this conclusion is so elementary that it might be characterized as “environmental science 101,” to use the vernacular. Johnson et al. (2017) put it this way:

“All life on planet Earth (i.e., the biosphere) exists at the intersections among the three abiotic spheres, i.e., the atmosphere, hydrosphere, and lithosphere, and is dependent on their interplay for continued existence over time. These four spheres are all interrelated in a huge variety of ways, in a planet-wide system of energy flow and the cycling of materials referred to as the ecosystem.”

Again, this idea is fundamental to the study of environmental science and should not be considered as new to the above-cited study of planetary boundaries. Nevertheless, the speed at which these planet-wide changes are occurring should give any person who reaches conclusions based on demonstrable evidence pause. The first report discussed above appeared in 2009, the second in 2015, and the third in 2023...a span of 14 years. Therefore, one of the most important questions facing all of humanity at this juncture is how much more time needs to be poorly used to the point that the remaining planetary boundaries will be breached.

The herpetofauna of Chihuahua, Mexico

Table 7. Distributional and conservation status measures for members of the herpetofauna of Chihuahua, Mexico. Distributional status: SE = endemic to state of Chihuahua; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. The numbers suffixed to the NE category signify the distributional categories developed by Wilson et al. (2017) and implemented in the taxonomic list at the *Mesoamerican Herpetology* website (<http://mesoamericanherpetology.com>), as follows: 3 = species distributed only in Mexico and the United States; 6 = species ranging from Mexico to South America; 7 = species ranging from the United States to Central America; and 8 = species ranging from the United States to South America. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = Endemic to Mexico; ** = Endemic to Chihuahua; *** = Non-native. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxon	Distributional status	Environmental Vulnerability Category (score)	IUCN categorization	SEMARNAT status
<i>Anaxyrus cognatus</i>	NE3	L (9)	LC	NS
<i>Anaxyrus debilis</i>	NE3	L (7)	LC	Pr
<i>Anaxyrus mexicanus</i> *	CE	M (13)	LC	NS
<i>Anaxyrus punctatus</i>	NE3	L (5)	LC	NS
<i>Anaxyrus speciosus</i>	NE3	M (12)	LC	NS
<i>Anaxyrus woodhousii</i>	NE3	M (10)	LC	NS
<i>Incilius alvarius</i>	NE3	M (11)	LC	NS
<i>Incilius mazatlanensis</i> *	CE	M (12)	LC	NS
<i>Incilius mccoysi</i> *	CE	H (14)	LC	NS
<i>Incilius occidentalis</i> *	CE	M (11)	LC	NS
<i>Rhinella horribilis</i>	NE7	L (3)	NE	NS
<i>Craugastor augusti</i>	NE3	L (8)	LC	NS
<i>Craugastor tarahumaraensis</i> *	CE	H (17)	LC	Pr
<i>Eleutherodactylus interorbitalis</i> *	CE	H (15)	LC	Pr
<i>Eleutherodactylus marnockii</i>	NE3	M (11)	LC	NS
<i>Dryophytes arenicolor</i>	NE3	L (7)	LC	NS
<i>Dryophytes wrightorum</i>	NE3	L (9)	LC	NS
<i>Smilisca baudinii</i>	NE7	L (3)	LC	NS
<i>Tlalocohyla smithi</i> *	CE	M (11)	LC	NS
<i>Gastrophryne mazatlanensis</i>	NE3	L (8)	LC	NS
<i>Gastrophryne olivacea</i>	NE3	L (9)	LC	Pr
<i>Hypopachus variolosus</i>	NE7	L (4)	LC	NS
<i>Agalychnis dacnicolor</i> *	CE	M (13)	LC	NS
<i>Lithobates berlandieri</i>	NE3	L (7)	LC	Pr
<i>Lithobates catesbeianus</i> ***	NN	—	—	—
<i>Lithobates chiricahuensis</i>	NE3	M (11)	VU	A
<i>Lithobates cora</i> *	CE	M (13)	LC	NS
<i>Lithobates lemosespinali</i> *	CE	H (14)	DD	NS
<i>Lithobates magnaocularis</i> *	CE	M (12)	LC	NS
<i>Lithobates pustulosus</i> *	CE	L (9)	LC	Pr
<i>Lithobates tarahumarae</i>	NE3	L (8)	VU	NS
<i>Lithobates yavapaiensis</i>	NE3	M (12)	LC	Pr
<i>Scaphiopus couchii</i>	NE3	L (3)	LC	NS
<i>Spea bombifrons</i>	NE3	M (10)	LC	NS
<i>Spea multiplicata</i>	NE3	L (6)	LC	NS
<i>Ambystoma mavortium</i>	NE3	M (10)	LC	NS
<i>Ambystoma rosaceum</i> *	CE	H (14)	LC	Pr
<i>Ambystoma silvense</i> *	CE	H (14)	DD	NS
<i>Ambystoma velasci</i> *	CE	M (10)	LC	Pr
<i>Isthmura sierraoccidentalis</i> *	CE	H (17)	VU	NS
<i>Barisia ciliaris</i> *	CE	H (15)	LC	NS
<i>Barisia imbricata</i> *	CE	H (14)	LC	Pr
<i>Barisia levicollis</i> *	CE	H (15)	DD	Pr
<i>Elgaria kingii</i>	NE3	M (10)	LC	Pr
<i>Gerrhonotus infernalis</i>	NE3	M (13)	LC	NS
<i>Norops nebulosus</i> *	CE	M (13)	LC	NS
<i>Crotaphytus collaris</i>	NE3	M (13)	LC	A
<i>Gambelia wislizenii</i>	NE3	M (13)	LC	Pr
<i>Coleonyx brevis</i>	NE3	H (14)	LC	Pr
<i>Hemidactylus turcicus</i> ***	NN	—	LC	—
<i>Heloderma horridum</i> *	CE	M (11)	LC	A
<i>Heloderma suspectum</i>	NE3	H (15)	NT	A
<i>Ctenosaura macrolopha</i> *	CE	H (19)	LC	NS
<i>Cophosaurus texanus</i>	NE3	H (14)	LC	A
<i>Holbrookia approximans</i> *	CE	H (14)	NE	NS
<i>Holbrookia elegans</i>	NE3	M (13)	LC	NS
<i>Holbrookia maculata</i>	NE3	M (10)	LC	NS
<i>Phrynosoma cornutum</i>	NE3	M (11)	LC	NS
<i>Phrynosoma hernandesi</i>	NE3	M (13)	LC	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Chihuahua, Mexico. Distributional status: SE = endemic to state of Chihuahua; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. The numbers suffixed to the NE category signify the distributional categories developed by Wilson et al. (2017) and implemented in the taxonomic list at the *Mesoamerican Herpetology* website (<http://mesoamericanherpetology.com>), as follows: 3 = species distributed only in Mexico and the United States; 6 = species ranging from Mexico to South America; 7 = species ranging from the United States to Central America; and 8 = species ranging from the United States to South America. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = Endemic to Mexico; ** = Endemic to Chihuahua; *** = Non-native. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxon	Distributional status	Environmental Vulnerability Category (score)	IUCN categorization	SEMARNAT status
<i>Phrynosoma modestum</i>	NE3	M (12)	LC	NS
<i>Phrynosoma orbiculare</i> *	CE	M (12)	LC	A
<i>Sceloporus albiventris</i> *	CE	H (16)	NE	NS
<i>Sceloporus clarkii</i>	NE3	M (10)	LC	NS
<i>Sceloporus cowlesi</i>	NE3	M (13)	NE	NS
<i>Sceloporus jarrovi</i>	NE3	M (11)	LC	NS
<i>Sceloporus lemosespinali</i> *	CE	H (16)	DD	NS
<i>Sceloporus magister</i>	NE3	L (9)	LC	NS
<i>Sceloporus merriami</i>	NE3	M (13)	LC	NS
<i>Sceloporus nelsoni</i> *	CE	M (13)	LC	NS
<i>Sceloporus poinsettii</i>	NE3	M (12)	LC	NS
<i>Sceloporus slevini</i>	NE3	M (11)	LC	NS
<i>Sceloporus virgatus</i>	NE3	H (15)	LC	NS
<i>Uma paraphygas</i> *	CE	H (17)	NT	P
<i>Urosaurus bicarinatus</i> *	CE	M (12)	LC	NS
<i>Urosaurus ornatus</i>	NE3	M (10)	LC	NS
<i>Uta stansburiana</i>	NE3	L (7)	LC	A
<i>Phyllodactylus saxatilis</i> *	CE	H (16)	NE	NS
<i>Plestiodon bilineatus</i> *	CE	M (13)	NE	NS
<i>Plestiodon callicephalus</i>	NE3	M (12)	LC	NS
<i>Plestiodon multilineatus</i> **	SE	H (16)	DD	Pr
<i>Plestiodon multivirgatus</i>	NE3	H (14)	LC	Pr
<i>Plestiodon obsoletus</i>	NE3	M (11)	LC	NS
<i>Plestiodon parviauriculatus</i> *	CE	H (15)	DD	Pr
<i>Plestiodon tetragrammus</i>	NE3	M (12)	LC	NS
<i>Aspidoscelis costata</i> *	CE	M (11)	LC	Pr
<i>Aspidoscelis exsanguis</i>	NE3	H (14)	LC	NS
<i>Aspidoscelis gularis</i>	NE3	L (9)	LC	NS
<i>Aspidoscelis inornata</i>	NE3	H (14)	LC	NS
<i>Aspidoscelis marmorata</i>	NE3	H (14)	NE	NS
<i>Aspidoscelis neomexicanus</i>	NE3	H (15)	LC	Pr
<i>Aspidoscelis sonora</i>	NE3	M (13)	LC	NS
<i>Aspidoscelis tessellata</i>	NE3	H (14)	LC	NS
<i>Aspidoscelis uniparens</i>	NE3	H (15)	LC	NS
<i>Boa sigma</i> *	CE	H (15)	NE	NS
<i>Arizona elegans</i>	NE3	L (5)	LC	NS
<i>Bogertophis subocularis</i>	NE3	H (14)	LC	NS
<i>Conopsis nasus</i> *	CE	M (11)	LC	NS
<i>Drymarchon melanurus</i>	NE6	L (6)	LC	NS
<i>Drymobius margaritiferus</i>	NE8	L (6)	LC	NS
<i>Gyalopion canum</i>	NE3	L (9)	LC	NS
<i>Gyalopion quadrangulare</i>	NE3	M (11)	LC	Pr
<i>Lampropeltis alterna</i>	NE3	H (14)	LC	A
<i>Lampropeltis knoblochi</i>	NE3	M (10)	LC	NS
<i>Lampropeltis polyzona</i> *	CE	L (7)	LC	NS
<i>Lampropeltis splendida</i>	NE3	M (12)	LC	NS
<i>Leptophis diplotropis</i> *	CE	H (14)	LC	A
<i>Masticophis bilineatus</i>	NE3	M (11)	LC	NS
<i>Masticophis flagellum</i>	NE3	L (8)	LC	A
<i>Masticophis mentovarius</i>	NE6	L (6)	LC	A
<i>Masticophis taeniatus</i>	NE3	M (10)	LC	NS
<i>Mastigodryas cliftoni</i> *	CE	H (14)	DD	NS
<i>Opheodrys vernalis</i>	NE3	H (14)	LC	NS
<i>Oxybelis microphthalmus</i>	NE3	M (11)	NE	NS
<i>Pantherophis bairdi</i>	NE3	H (15)	LC	NS
<i>Pantherophis emoryi</i>	NE3	M (13)	LC	NS
<i>Pituophis catenifer</i>	NE3	L (9)	LC	NS
<i>Pituophis deppei</i> *	CE	H (14)	LC	A
<i>Rhinocheilus lecontei</i>	NE3	L (8)	LC	NS

The herpetofauna of Chihuahua, Mexico

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Chihuahua, Mexico. Distributional status: SE = endemic to state of Chihuahua; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. The numbers suffixed to the NE category signify the distributional categories developed by Wilson et al. (2017) and implemented in the taxonomic list at the *Mesoamerican Herpetology* website (<http://mesoamericanherpetology.com>), as follows: 3 = species distributed only in Mexico and the United States; 6 = species ranging from Mexico to South America; 7 = species ranging from the United States to Central America; and 8 = species ranging from the United States to South America. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = Endemic to Mexico; ** = Endemic to Chihuahua; *** = Non-native. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxon	Distributional status	Environmental Vulnerability Category (score)	IUCN categorization	SEMARNAT status
<i>Salvadora bairdi</i> *	CE	H (15)	LC	Pr
<i>Salvadora deserticola</i>	NE3	H (14)	NE	NS
<i>Salvadora grahamiae</i>	NE3	M (10)	LC	NS
<i>Salvadora lineata</i>	NE3	M (11)	NE	NS
<i>Senticolis triaspis</i>	NE7	L (6)	LC	NS
<i>Sonora aemula</i> *	CE	H (16)	NT	Pr
<i>Sonora semianmulata</i>	NE3	L (5)	LC	NS
<i>Sympholis lippiens</i> *	CE	H (14)	DD	NS
<i>Tantilla atriceps</i>	NE3	M (11)	LC	A
<i>Tantilla cucullata</i>	NE3	M (12)	LC	NS
<i>Tantilla hobartsmithi</i>	NE3	M (11)	LC	NS
<i>Tantilla nigriceps</i>	NE3	M (11)	LC	NS
<i>Tantilla wilcoxi</i>	NE3	M (10)	LC	NS
<i>Tantilla yaquia</i>	NE3	M (10)	LC	NS
<i>Trimorphodon tau</i> *	CE	M (13)	LC	NS
<i>Trimorphodon wilkinsonii</i>	NE3	H (15)	LC	A
<i>Diadophis punctatus</i>	NE3	L (4)	LC	NS
<i>Geophis dugesii</i> *	CE	M (13)	LC	NS
<i>Heterodon kennerlyi</i>	NE3	M (11)	LC	NS
<i>Hypsiglena chlorophaea</i>	NE3	L (8)	LC	Pr
<i>Hypsiglena jani</i>	NE3	L (6)	LC	Pr
<i>Imantodes gemmistratus</i>	NE6	L (6)	LC	Pr
<i>Leptodeira splendida</i> *	CE	H (14)	LC	NS
<i>Rhadinaea hesperia</i> *	CE	M (10)	LC	Pr
<i>Rhadinaea laureata</i> *	CE	M (12)	LC	NS
<i>Tropidodipsas repleta</i> *	CE	H (17)	DD	NS
<i>Micruroides euryxanthus</i>	NE3	H (15)	LC	A
<i>Micrurus distans</i> *	CE	H (14)	LC	Pr
<i>Rena dugesii</i> *	CE	H (14)	NE	NS
<i>Rena dulcis</i>	NE3	M (13)	LC	NS
<i>Rena humilis</i>	NE3	L (8)	LC	NS
<i>Rena segregata</i>	NE3	L (8)	NE	NS
<i>Nerodia erythrogaster</i>	NE3	M (11)	LC	A
<i>Storeria storerioides</i> *	CE	M (11)	LC	NS
<i>Thamnophis cyrtopsis</i>	NE7	L (7)	LC	A
<i>Thamnophis elegans</i>	NE3	H (14)	LC	A
<i>Thamnophis eques</i>	NE3	L (8)	LC	A
<i>Thamnophis errans</i> *	CE	H (16)	LC	NS
<i>Thamnophis marcianus</i>	NE7	M (10)	LC	A
<i>Thamnophis melanogaster</i> *	CE	H (15)	EN	A
<i>Thamnophis sirtalis</i>	NE3	H (14)	LC	Pr
<i>Thamnophis unilabialis</i> *	CE	H (16)	NE	NS
<i>Thamnophis validus</i> *	CE	M (12)	NE	NS
<i>Virgotyphlops braminus</i> ***	NN	—	LC	—
<i>Agkistrodon bilineatus</i>	NE4	M (11)	NT	Pr
<i>Agkistrodon laticinctus</i>	NE3	H (15)	LC	NS
<i>Crotalus atrox</i>	NE3	L (9)	LC	Pr
<i>Crotalus basiliscus</i> *	CE	H (16)	LC	Pr
<i>Crotalus lepidus</i>	NE3	M (12)	LC	Pr
<i>Crotalus molossus</i>	NE3	L (8)	LC	Pr
<i>Crotalus ornatus</i>	NE3	M (13)	NE	NS
<i>Crotalus pricei</i>	NE3	H (14)	LC	Pr
<i>Crotalus scutulatus</i>	NE3	M (11)	LC	Pr
<i>Crotalus viridis</i>	NE3	M (12)	LC	Pr
<i>Crotalus willardi</i>	NE3	M (13)	LC	Pr
<i>Chrysemys picta</i>	NE3	H (14)	LC	A
<i>Terrapene nelsoni</i> *	CE	H (18)	DD	Pr
<i>Terrapene ornata</i>	NE3	H (15)	NT	Pr
<i>Trachemys gaigeae</i>	NE3	H (18)	VU	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Chihuahua, Mexico. Distributional status: SE = endemic to state of Chihuahua; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. The numbers suffixed to the NE category signify the distributional categories developed by Wilson et al. (2017) and implemented in the taxonomic list at the *Mesoamerican Herpetology* website (<http://mesoamericanherpetology.com>), as follows: 3 = species distributed only in Mexico and the United States; 6 = species ranging from Mexico to South America; 7 = species ranging from the United States to Central America; and 8 = species ranging from the United States to South America. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = Endemic to Mexico; ** = Endemic to Chihuahua; *** = Non-native. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxon	Distributional status	Environmental Vulnerability Category (score)	IUCN categorization	SEMARNAT status
<i>Rhinoclemmys pulcherrima</i>	NE4	L (8)	NE	A
<i>Kinosternon durangoense</i> *	CE	H (16)	DD	NS
<i>Kinosternon flavescens</i>	NE3	M (12)	LC	NS
<i>Kinosternon hirtipes</i>	NE3	M (10)	LC	Pr
<i>Kinosternon integrum</i> *	CE	M (11)	LC	Pr
<i>Kinosternon sonoriense</i>	NE3	H (14)	NT	P
<i>Gopherus evgoodei</i> *	CE	H (18)	VU	NS
<i>Gopherus flavomarginatus</i> *	CE	H (19)	CR	P
<i>Apalone spinifera</i>	NE3	H (15)	LC	Pr

Table 8. Summary of the distributional status of herpetofaunal families in Chihuahua, Mexico.

Family	Number of species	Distributional status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
Bufonidae	11	7	4	—	—
Craugastoridae	2	1	1	—	—
Eleutherodactylidae	2	1	1	—	—
Hylidae	4	3	1	—	—
Microhylidae	3	3	—	—	—
Phyllomedusidae	1	—	1	—	—
Ranidae	9	4	4	—	1
Scaphiopodidae	3	3	—	—	—
Subtotal	35	22	12	—	1
Ambystomatidae	4	1	3	—	—
Plethodontidae	1	—	1	—	—
Subtotal	5	1	4	—	—
Total	40	23	16	—	1
Anguidae	5	2	3	—	—
Anolidae	1	—	1	—	—
Crotaphytidae	2	2	—	—	—
Eublepharidae	1	1	—	—	—
Gekkonidae	1	—	—	—	1
Helodermatidae	2	1	1	—	—
Iguanidae	1	—	1	—	—
Phrynosomatidae	23	16	7	—	—
Phyllodactylidae	1	—	1	—	—
Scincidae	7	4	2	1	—
Teiidae	9	8	1	—	—
Subtotal	53	34	17	1	1
Boidae	1	—	1	—	—
Colubridae	40	31	9	—	—
Dipsadidae	10	5	5	—	—
Elapidae	2	1	1	—	—
Leptotyphlopidae	4	3	1	—	—
Natricidae	11	6	5	—	—
Typhlopidae	1	—	—	—	1
Viperidae	11	10	1	—	—
Subtotal	80	56	23	—	1
Emydidae	4	3	1	—	—
Geoemydidae	1	1	—	—	—
Kinosternidae	5	3	2	—	—
Testudinidae	2	—	2	—	—
Trionychidae	1	1	—	—	—
Subtotal	13	8	5	—	—
Total	146	98	45	1	2
Sum total	186	121	61	1	3

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No. 21. *Crotaphytus collaris* (Say, 1823). The Eastern Collared Lizard is distributed from “Missouri to Arizona, in the United States, and in Mexico in the area between the Sierra Madre Occidental and Sierra Madre Oriental to San Luis Potosí, where it has been recorded in the western half of the state” (Lemos-Espinal and Dixon 2013: 104–105). This individual was photographed at Charco de Peña, in the municipality of Julimes, Chihuahua, where it was in the shade while perched on a large rock. Wilson et al. (2013a) calculated the EVS of this species as 13, placing it at the upper limit of the medium vulnerability category. The IUCN determined its conservation status as Least Concern, but it was assessed as Threatened by SEMARNAT. *Photo by Ana B. Gatica-Colima.*



No. 22. *Gambelia wislizenii* (Baird and Girard, 1852). The Longnose Leopard Lizard “is widely distributed in western North America, ranging from Oregon and Idaho through the Great Basin and deserts of the southwestern United States southward into northern Mexico... In Mexico, *G. wislizenii* occurs west of the Sierra Madre Occidental in northeastern Baja California...and in western Sonora...This lizard is also known from Isla Tiburón...East of the Sierra Madre Occidental, *G. wislizenii* ranges into northern Chihuahua, and in apparently disjunct populations further south through the southern half of Coahuila and adjacent regions of southeastern Chihuahua and northwestern Durango...” (Heimes 2022: 54). This individual was photographed under the cover of mesquite, east of Rancho El Lobo, in Juárez, Chihuahua. Wilson et al. (2013a) determined the EVS of this lizard as 13, placing it at the upper limit of the medium vulnerability category. The IUCN ascertained its conservation status as Least Concern, and it is regarded as a species of Special Protection by SEMARNAT. *Photo by Ana B. Gatica-Colima.*



No. 23. *Ctenosaura maculata* Smith, 1972. The Sonoran Spiny-tailed Iguana is distributed in “Sonora and adjacent areas of Chihuahua and Sinaloa” (Heimes 2022: 146). This juvenile was photographed on a rock on the side of a dirt road traversing a steep hill. The surrounding vegetation consisted of lowland forest with mosses and grasses near the small town of Batopilas, Chihuahua. Wilson et al. (2013a) judged its EVS as 19, placing it in the upper portion of the high vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. *Photo by Jesús M. Martínez-Calderas.*



No. 24. *Cophosaurus texanus* Troschel, 1852. The distribution of the Greater Earless Lizard “extends from southeastern Arizona to western Texas, in the United States, and in Mexico from northeastern Sonora southward through eastern Chihuahua and into San Luis Potosí” (Lemos-Espinal and Dixon 2013: 117–118). This individual was photographed in scrub vegetation alongside a road to Bismark mine, in Ascensión, Chihuahua. Wilson et al. (2013a) calculated the EVS of this species as 14, placing it at the lower limit of the high vulnerability category. The IUCN has assessed its conservation status as Least Concern, but this species is considered as Threatened by SEMARNAT. *Photo by Eric Centenero-Alcalá.*

Table 9. Summary of the distributional categories of the herpetofaunal families in Chihuahua, Mexico, that contain non-endemic species. The categorizations are as follows: MXUS = species distributed only in Mexico and the United States (except for a few perhaps found in Canada); MXCA = species found only in Mexico and Central America; MXSA = species ranging from Mexico to South America; USCA = species ranging from the United States to Central America (except for a few perhaps found in the Antilles); and USSA = species ranging from the United States to South America.

Family	Number of non-endemic species	Distributional status				
		MXUS Species (3)	MXCA Species (4)	MXSA Species (6)	USCA Species (7)	USSA Species (8)
Bufonidae	7	6	—	—	1	—
Craugastoridae	1	1	—	—	—	—
Eleutherodactylidae	1	1	—	—	—	—
Hylidae	3	2	—	—	1	—
Microhylidae	3	2	—	—	1	—
Ranidae	5	4	1	—	—	—
Scaphiopodidae	3	3	—	—	—	—
Subtotal	23	19	1	—	3	—
Ambystomatidae	1	1	—	—	—	—
Subtotal	1	1	—	—	—	—
Total	24	20	1	—	3	—
Anguidae	2	2	—	—	—	—
Crotaphytidae	2	2	—	—	—	—
Eublepharidae	1	1	—	—	—	—
Helodermatidae	1	1	—	—	—	—
Phrynosomatidae	16	16	—	—	—	—
Scincidae	4	4	—	—	—	—
Teiidae	7	7	—	—	—	—
Subtotal	33	33	—	—	—	—
Colubridae	31	27	—	2	1	1
Dipsadidae	5	4	—	1	—	—
Elapidae	1	1	—	—	—	—
Leptotyphlopidae	3	3	—	—	—	—
Natricidae	6	4	—	—	2	—
Viperidae	10	9	1	—	—	—
Subtotal	56	48	1	3	3	1
Emydidae	3	3	—	—	—	—
Geoemydidae	1	—	1	—	—	—
Kinosternidae	3	3	—	—	—	—
Trionychidae	1	1	—	—	—	—
Subtotal	8	7	1	—	—	—
Total	97	88	2	3	3	1
Sum total	121	108	3	3	6	1

Accordingly, it is important to understand that the protection of organismic populations through the erection and maintenance of NPAs is only a relatively small part of what humans need to do to help maintain the biosphere as a functional component of a sustainable world, but this action is something that conservation biologists can do to help protect the biosphere. In the context of this paper, we identify the major threats to the herpetofauna of Chihuahua, as follows: land conversion and habitat loss; improper management of water quality and quantity; invasive species; climate change; fires and illegal logging; illegal trade; infectious diseases and parasites; on and off-road activities; mining; solid waste pollution; consumption of amphibians and reptiles;

agriculture and livestock grazing; fear and/or confusion; and miscellaneous threats.

Land conversion and habitat loss (Figs. 14–16)

The inhabitants of the Raramuri communities of Ejido Arroyo de La Cabeza, Comunidad Laguna, Ejido Panalachi, and Ejido Sisoguichi, in the municipality of Bocoyna, recognize deforestation and clandestine logging as major environmental problems, in addition to the erosion of lands (WWF 2008). Ceballos et al. (2010) demonstrated a large decline in the abundance of vertebrates across all taxonomic groups (mammals, native and migratory birds, and reptiles). The

Table 10. Comparison of the numbers of endemic, non-endemic, and non-native species, and the percentage of endemism for Chihuahua, Mexico, and the surrounding Mexican states. The percentage of endemism is calculated as the number of endemic species/total native herpetofauna. Data in the table are from this study for Chihuahua, Lemos-Espinal et al. (2019) for Sonora, Lemos-Espinal et al. (2020) for Sinaloa, Lemos-Espinal et al. (2018) for Durango, and Lazcano et al. (2019) for Coahuila, along with the calculations pertaining to the percentage of endemism.

State	Total herpetofauna	Endemic species	% of endemism	Non-endemic species	Non-native species
Chihuahua	183	59	32.2	121	3
Sonora	200	68	35.2	125	7
Sinaloa	159	82	52.9	73	4
Durango	156	72	47.1	81	3
Coahuila	143	40	28	100	3



Fig. 14. The removal of vegetation from the arid lands of northern Chihuahua exposes an increasing amount of soil erosion, and dust storms occur in Ascensión, Chihuahua. *Photo by Ana Gatica-Colima.*



Fig. 15. The logging of trees creates an environmental problem even at a low scale, as it transforms the landscape and habitat of vertebrates. *Photo by Ana Gatica-Colima.*

55,000 ha Prairie Dog colony complex has declined by 73% since 1988. It has become increasingly fragmented, and densities have shown a precipitous decline over the years, from an average of 25/ha in 1988 to 2/ha in 2004.

The PMARP (2012), a master plan by the regional alliance for the conservation of grasslands in the Chihuahuan Desert, identified 10 major problems or necessities for conserving the Chihuahuan Desert grasslands in the states of Zacatecas and Chihuahua, one of which is land use conversion. In particular, these problems are significant in Chihuahua, as it is one of the states in this ecoregion that has been impacted by a large scale of illegal changes in land conversion.

Munguia-Vega et al. (2013) studied the localized extinction of an arboreal desert lizard (*Urosaurus nigricaudus*) caused by fragmentation, suggesting that limited dispersal, coupled with an inability to use a homogeneous and hostile matrix without vegetation and shade, could result in frequent time-delayed extinctions of small ectotherms in highly fragmented desert landscapes, particularly when considering an increase in the risk of overheating and a reduction in dispersal potential induced by global warming. Lavín-Murcio et al. (2014) selected *Ambystoma rosaceum*, *Craugastor tarahumarensis*,

Crotalus viridis, and *Gopherus flavomarginatus* as targets for immediate action to avoid their disappearance in Chihuahua, pointing out the destruction of habitats as one of the more harmful threats to amphibians and reptiles.

The grassland areas of the Chihuahuan Desert Ecoregion are undergoing a large-scale transformation, principally due to expanding agriculture, urbanization, energy development, and desertification (Pool et al. 2014).

The results of a geospatial analysis of land use and water in the peri-urban area of Ciudad Cuauhtémoc, Chihuahua, indicate that the processes of changes in land use and vegetation cover occur within a context of high competition for water among the various users (Díaz-Cervantes et al. 2014).

Importantly, we need to conserve the natural habitats in the Sierra de Juárez, Juárez, Chihuahua, because of the potential risk and threats to biodiversity. The construction of the “Camino Real” road and increasing urban development are present in the Sierra de Juárez (Gatica-Colima et al. 2014a; Fernández-López and Lavín-Murcio 2016).

Clarke-Crespo et al. (2017) used a multi-objective method to assess the quality of grasslands in the northern



No. 25. *Holbrookia maculata* Girard, 1851. The Lesser Earless Lizard “inhabits much of the Great Plains of North America, ranging from South Dakota south to central and western Texas, thence westward into Arizona, New Mexico and northern Chihuahua” (Heimes 2022: 188). This individual was photographed in sand dunes (médanos) at Rancho El Lobo, in Juárez, Chihuahua. Wilson et al. (2013a) determined its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN determined its conservation status as Least Concern, and this species is not listed by SEMARNAT. Photo by Eric Centenero-Alcalá.



No. 26. *Phrynosoma cornutum* (Harlan, 1824). The distribution of the Texas Horned Lizard “extends from Kansas southwestward to southeastern Arizona, then southward through all of northern Mexico east of the Sierra Madre Occidental to Durango, and eastward through all of Coahuila and Nuevo León, Tamaulipas, and disparate localities in San Luis Potosí” (Lemos-Espinal and Dixon 2013: 119–120). This adult individual was photographed in loam-gravel substrate associated with a downhill slope containing microphyllous desert scrub vegetation (with *Larrea tridentata* and *Opuntia leptocaulis*), from where it fled to seek refuge. This location lies south of Sierra Samalayuca, in Juárez, Chihuahua. Wilson et al. (2013a) established its EVS as 11, placing it in the middle of the medium vulnerability category. The IUCN assessed its conservation status as Least Concern, and this species is not listed by SEMARNAT. Photo by Jesús M. Martínez-Calderas.



No. 27. *Phrynosoma hernandesi* Girard, 1858. The Greater Short-horned Lizard “ranges from central Utah and southwestern Colorado southward to northeastern Sonora and adjacent northwestern Chihuahua” (Heimes 2022: 218). This individual was photographed in grassland habitat in Namiquipa, Chihuahua, and the disturbance caused it to start squirting blood from the ducts in its eyes. Wilson et al. (2013a) determined its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN judged its conservation status as Least Concern, and this species is not listed by SEMARNAT. Photo by Sebastian Ochoa Rodríguez.



No. 28. *Phrynosoma modestum* Girard, 1852. The distribution of the Round-tailed Horned Lizard “extends from southeastern Arizona to western Texas and southeastern Colorado, in the United States, southward in Mexico through Chihuahua, east of the Sierra Madre Occidental (except for the northwestern portion) to San Luis Potosí...” (Lemos-Espinal and Dixon 2013: 121). This individual was photographed in rocky substrate along the side of a dirt road to El Marro Canyon, an ecological park in Chihuahua. Wilson et al. (2013a) calculated its EVS as 12, placing it in the middle portion of the medium vulnerability category. The IUCN has assessed the conservation status of this lizard as Least Concern, and this species is not listed by SEMARNAT. Photo by Ana B. Gatica-Colima.



Fig. 16. The removal of the natural vegetation can produce land erosion, Bocoyna, Chihuahua. Chihuahua. Photo by Ana Gatica-Colima.

Chihuahuan Desert, and demonstrated that the values of the 14-patch metrics confirmed that these grasslands are undergoing intense fragmentation in the Chihuahuan Desert landscape, and also that the grassland ecosystems are in a highly vulnerable state.

Reyes-Gómez et al. (2020) diagnosed three aquifers in the Chihuahuan Desert, the Tabalaopa Aldama (TA), San Diego (SD), and Laguna de Hormigas (LH), and concluded that the types of land use change identified for a 19-year period (1993–2012) show a significant loss of primary and secondary vegetation cover represented by desert scrub and grassland (480.24 km² in total; 11.7% in the TA aquifer, 12.1% in the SD aquifer, and 76.2% in the Laguna de Hormigas LH aquifer). These losses in vegetation cover produced significant increases in the agricultural frontier (356.9 km² in total; 0.15% in TA, 14.9% in SD and 85% in LH), and in the urban sprawl (54.93 km² in total; 8% in SD, 92% in TA and 0% in LH). The largest area of primary and secondary vegetation cover transformed to urban use occurred in the southwestern portion of the TA aquifer, in the neighborhoods in the southern periphery of the city of Chihuahua, and showed little representation in the SD and LH aquifers.

The Ramsar site along Río San Pedro-Meoqui, Chihuahua (No. 2047) is an area that suffers consistent anthropogenic disturbances, such as the extraction of stone material from the riverbed, as recorded in 2015 (see Loredó-Varela and Hernández-Escudero 2021).

Improper management of water quality and quantity (Figs. 17–18)

An overexploitation of aquifers occurs in Chihuahua (Chávez-Rodríguez et al. 2007). In a study of the aquifer

conditions in the Laguna Bustillos Basin (Chihuahua, Mexico) in recent decades (1991–2012), Alatorre et al. (2019) noted that the least favorable conditions for furrow irrigation were in Mennonite properties.

About 90% of the surface water and 84% of the pumped groundwater are used for irrigation in the state of Chihuahua (CONAGUA 2020).

More recently, Rentería-Villalobos et al. (2022) evaluated the impact of climate variability on the sustainability of the transboundary water supply in Chihuahua, Mexico. They sampled three reservoirs (Boquilla, Francisco I. Madero, and Granero), and concluded that climate variability and temperature increases will amplify the cycles of supply and demand, which will undermine sustainability, mitigation, and management strategies.

The residents of the Raramuri communities in Ejido Arroyo de La Cabeza, Comunidad Laguna, and Ejido Sisoguichi, in the municipality of Bocoyna, recognize water pollution as a principal environmental problem (WWF 2008).

Gutiérrez et al. (2008) suggested that human activities associated with the Conchos River (wastewater discharges and waste products from agriculture and industrial activities) are the source of arsenic (As) contamination in the San Pedro River. Gutiérrez et al. (2009) also found that sediments from the Río Conchos Basin in northern Mexico contained arsenic levels that exceeded the guideline value (22 mg kg⁻¹) for agricultural soils.

According to Gallo-Reynoso et al. (2020), before entering Pegüis Canyon, the waters of the Conchos River flow through vast agricultural and urban areas in the central part of the state of Chihuahua, where numerous dams divert water to irrigation channels and the river receives urban and agricultural wastewater.

Ríos-Arana et al. (2007) commented that the high conductivity values measured in September and October might reflect reduced flows in the Río Bravo at the end of the irrigation season.

The Raramuri communities Ejido Arroyo de La Cabeza, Comunidad Laguna, and Ejido Sisoguichi from the municipality of Bocoyna, also recognize reduced flow in rivers and water scarcity as principal environmental problems (WWF 2008).

Between the first visit to a section of the Río Florido near the Aguila Dam, Coronado, in August of 2000, and a second in June of 2013, Gatica-Colima (2017) noticed that the flow of the river was less than in previous years. The local people indicated that the water was being managed upstream.

Invasive species (Fig. 19)

The Mediterranean Gecko (*Hemidactylus turcicus*) was reported from Camargo, Chihuahua (Lemos-Espinal and Smith 2007), and later from Juárez, Chihuahua (Gatica-Colima et al. 2009). This species is well established and often seen on the walls of buildings at the Universidad Autónoma de Ciudad Juárez campus (AGC, pers. obs.).

In Chihuahua, the American Bullfrog (*Lithobates catesbeianus*) has been reported from the municipalities of Ascensión, Janos, Juárez, and Nuevo Casas Grandes



Fig. 17. The constant irrigation of crops in the desert drives the excessive extraction of subterranean water. *Photo by Ana Gatica-Colima.*



Fig. 18. The reduction of water levels in the Janos reservoir reveals the demand for water over the years. *Photo by Ana Gatica-Colima.*

(Lemos-Espinal and Smith 2007). This species also appears to have an established population in Presa Rosetilla, in the municipality of Saucillo (Gatica-Colima et al. 2014b), and Carbajal-Márquez et al. (2014) reported it from the Río Chuvistar. There is evidence of *L. catesbeianus* preying on the Green Toad (*Anaxyrus debilis*) and on a species of gartersnake (*Thamnophis*) (Fig. 19) in the municipality of Janos in northwestern Chihuahua (Ramos-Guerra and Gatica-Colima 2014). According to the NOM-059-SEMARNAT-2010, *A. debilis* is categorized as a species of Special Protection. Additionally, a population of *L. catesbeianus* from southeastern Texas ($n = 45$) was found to have a component parasite community consisting of three nematode species, six trematodes, and a single acanthocephalan, with a prevalence of 93% (Yoder and Gomez 2007).

Mendoza-Almeralla et al. (2015) undertook a review of chytridiomycosis in amphibians from Mexico and recognized the introduction and displacement of the American Bullfrog as an important factor for spreading the causative pathogen *Batrachochytrium dendrobatidis* (Bd). Although Bd has not been detected in Chihuahuan amphibians, Hernández-Martínez et al. (2019) provided

the first records of Bd in anurans from the Nazas-Aguanaval basin in the states of Coahuila and Durango. They reported detecting Bd in *Lithobates berlandieri* and *L. catesbeianus*, as well as in *Anaxyrus cognatus*, *A. debilis*, *A. punctatus*, and *Gastrophryne olivacea*, all of which are found in Chihuahua. Importantly, a survey needs to be conducted in southern Chihuahua to see if this pathogen can be detected in anurans from the state.

In their review of the introduced herpetofauna of Mexico and Central America, González-Sánchez et al. (2021) listed two invasive species for Chihuahua, i.e., the Mediterranean Gecko (*Hemidactylus turcicus*) and the American Bullfrog (*Lithobates catesbeianus*). Another invasive species in Chihuahua is *Indotyphlops* [formerly in the genus *Virgotyphlops*] *braminus* (Cruz Elizalde et al. 2022). Initially, Carbajal-Márquez et al. (2015) reported this species as a new state record, as this exotic snake was found in a garden in the city of Chihuahua. According to Wallach (2020b), Chihuahua is the 26th place in Mexico where this species has been recorded as of 2015, as it was first recorded from Guerrero in 1891.

Other vertebrates are considered as potential threats to the environment and/or to amphibians and reptiles in Chihuahua, as noted below. In southern Chihuahua,

near the Águila Dam, in the municipality of Coronado, we encountered a large carapace of the Mexican Plateau Slider (*Trachemys gaigeae*). We observed large fish in the water, but there were no turtles basking on the logs, as we had seen in prior years (Gatica-Colima et al. 2017). The invasive fish species that we recorded from the Río Florido is the Common Carp (*Cyprinus carpio*) (<https://www.naturalista.mx/observations/56386114>). According to the Global Invasive Species Database (2013), *C. carpio* stirs up the sediments on the bottom in search of food, altering the habitat of native fish and other aquatic species. The rapid invasion of *C. carpio* was determined to be in a high-risk category, with an invasiveness value of 0.85 (CONABIO 2017).

According to Torres-Olave et al. (2018), Wild Boars (*Sus scrofa*) use the six main habitat types in Chihuahua, as follows: microphyll desert scrub (24,376.71 km²); rosetophyllous desert scrub (7,036.95 km²); natural grasslands (6,360.0 km²); halophytic grassland (4,017.75 km²); pine-oak forest (2,172 km²); and annual seasonal agriculture (1,946.84 km²). Although no studies have documented the threats of this species in Chihuahua, in the NPA of Sierra La Laguna in the state of Baja California Sur, Breceda et al. (2009) indicated that Wild Boars are known to have consumed a Desert Night Lizard (*Xantusia vigilis*) among other food items. Lastly, Soto-Cruz et al. (2014) documented the first record of the Monk Parakeet (*Myiopsitta monachus*) in the state of Chihuahua, and recorded active nests in the cities of Chihuahua and Delicias. The authors recommended follow-up population studies to assess the possible risks for the ecosystems in the region.

Climate change (Figs. 20–21)

Regarding this subject, Lara-Reséndiz et al. (2015) used data from field, laboratory, and modeling approaches for *Phrynosoma cornutum* and *P. modestum* at three contrasting sites in the Mexican Chihuahuan Desert (Mapimí, Durango; and Janos and Samalayuca, Chihuahua). The thermoregulatory indices suggested that both species thermoregulate effectively despite living in habitats of low thermal quality. Based on their measurements, if the air temperature rises, as predicted by climate models, the extinction model projects that *P. cornutum* will become locally extinct at 6% of the sites and *P. modestum* at 32% of the sites by the year 2050.

At the Sevilleta National Wildlife Refuge and Long-Term Ecological Research (LTER), New Mexico, USA, a short-term study of the influence of rainfall on microhabitat use by the Chihuahuan Spotted Whiptail (*Aspidoscelis exsanguis*) was conducted within Pinyon Pine (*Pinus edulis*) and One-seed Juniper (*Juniperus monosperma*) forest. The study showed that rainfall can influence lizard microhabitat use more than temperature in a Piñon Pine/Juniper woodland, and that the trees provide important refugia. The loss of Piñon Pine and Juniper trees from prolonged drought threatens to limit the amount of shade available for lizards in the future (Mason et al. 2016).



Fig. 19. Remains of a gartersnake (*Thamnophis* sp.) extracted from the stomach of an American Bullfrog (*Lithobates catesbeianus*), collected in Janos, Chihuahua, reveals the impact of this invasive species on native reptile fauna. Photo by Ana Gatica-Colima.

Aburto-Oropeza et al. (2018) noted that the effects of climate change will devastate biodiversity in the USA–Mexico border region, and suggested that by carefully selecting new montane preserves adjacent to desert and tropical forest habitats, and by implementing global controls on atmospheric carbon dioxide emissions, extinctions might be reduced to fewer than 11% of the species and to a single reptile family.

According to Pineda-Martínez (2020), the values from detection indexes associated with intense to extreme rainfall demonstrated a positive trend in the last decade. These trends are associated with an increase in sea surface temperature. The spatial distribution of these trends was positive in the SMO region and showed a negative anomaly in the core zone of the monsoon, but with an increase toward northern Mexico.

Lazcano et al. (2023) reported that on 18 September 2022, northwest of Juárez, they found what they considered to be a strange death for a Prairie Rattlesnake (*Crotalus viridis*). The authors noted that a strong rainstorm occurred between 1600 and 1900 h, which accumulated 9.8 mm of rain, followed by a heavy hailstorm between 1700 and 1715 h, which apparently caused its death. Further, they noted that climate change undoubtedly will enhance the occurrence of large hailstones, as warmer air containing more water vapor will enter the atmosphere, and that powerful storms with powerful updrafts will occur more frequently.

The Raramuri community of Ejido Panalachi, in the municipality of Bocoyna, recognizes fires as a principal environmental problem (WWF 2008). CONAFOR (2010) reported data for the 2009 average number of forest fires by state, and Chihuahua ranked 4th with 842, and 10th regarding the size of the area affected, with 10,703.87 ha.

Alva-Alvarez et al. (2018) evaluated the interaction between fires and landscape during the 2000–2010 period in Madera, Chihuahua, and identified 388 fire polygons covering 8,277 ha. On average 35 fires occur per year, with an annual fire rate of 9% and an average burn area of



Fig. 20. Climate change. Different rainfall patterns in recent years, such as reduced precipitation, have been causing extreme drought in the Rio Bravo/Rio Grande, Manuel Benavides, Chihuahua/Brewster Co. Texas. *Photo by Ana Gatica-Colima.*



Fig. 21. The removal of the natural vegetation cover for agricultural purposes triggers the burning of dry matter, a relatively new but common practice that generates air pollution. Ahumada, Chihuahua. *Photo by Ana Gatica-Colima.*

20.60 ha. The authors concluded that the burning of forests in Madera fragments landscapes with less connectivity.

According to the UNDRR (2021) the National Forestry Commission reported that 212,000 ha in all 32 states of Mexico were affected by forest fires in the year 2021; one of the most affected states was Chihuahua. Burning the vegetation cover that had been removed for agricultural purposes is a common practice in Chihuahua, and logging at a low scale is another environmental problem.

Bonello (2019) provided a warning about the increasing amount of illegal wood being extracted from Chihuahua. Lastly, Guerrero et al. (2000) noted that some populations of plant and animal species, including reptiles, have been depleted as the result of habitat loss in the Sierra Madre Occidental.

Illegal trade (Figs. 22–23)

Some reptile species for sale in pet stores in Juárez, Chihuahua, included the Spiny Softshell Turtle *Apalone spinifera* and the Ornate Box Turtle *Terrapene ornata*

according to a study conducted between April 1997 and July 1999, and both species are protected by law (Gatica-Colima and Bojorquez-Rangel 1999).

The herpetofaunal diversity of the Mexican Chihuahuan Desert Ecoregion (CDE) is among the highest in all the desert ecoregions. The CDE herpetofauna is composed of 29 species of amphibians and 131 species of reptiles. Approximately 63% ($n = 82$) of the 131 estimated reptile species found in the Mexican portion of the CDE (Cotera et al. 2001) are subject to some level of trade.

Fitzgerald et al. (2004) recorded rattlesnakes and Bolson Tortoises (*Gopherus flavomarginatus*) as reptiles that are subject to trade in the Chihuahuan Desert Ecoregion of Mexico. Garza-Almanza et al. (2010) listed 41 species of fauna that have been seized in Chihuahua, including the following reptiles: *Apalone spinifera*, *Boa constrictor*, *Crotalus durissus*, *Gopherus flavomarginatus*, *Heloderma suspectum*, *Iguana iguana*, *Kinosternon integrum*, *Kinosternon leucostomum*, *Masticophis flagellum*, *Python regius* (an introduced species), *Terrapene ornata*, and *Trachemys scripta*, all



Fig. 22. An increasing amount of illegal commerce is being detected in the region. Here, some anurans (Bufonidae) are being sold in a local market in northern Chihuahua. The demand for wildlife might be triggered by new beliefs from an increasing population of migrants along the border region. *Photo by Ramón I. Miramontes-Cinco.*



Fig. 23. Rattlesnake carcasses are sold at different prices (from 100 to 450 pesos) in a local market in northern Chihuahua. *Photo by Ramón I. Miramontes-Cinco.*



Fig. 24. Argasid ticks, one in the corner of the right eye (bottom left) and another in the first third of the body (can you find it?) of a Prairie Rattlesnake (*Crotalus viridis*) in Janos, Chihuahua. *Photo by Eduardo F. Macias-Rodríguez.*

of which except *P. regius* in a risk category according to SEMARNAT (2010).

Infectious diseases and parasites (Figs. 24–25)

Goldberg and Bursey (1991) found various helminth parasites in the lungs and gastrointestinal tracts of three species of toads from Arizona (USA): *Anaxyrus alvarius*, *Anaxyrus cognatus*, and *Scaphiopus couchii*. Although these helminths were found in anurans from the USA, these species of amphibians are abundant in Chihuahua after rains, primarily in the Sierras y Llanuras del Norte (SLN) province.

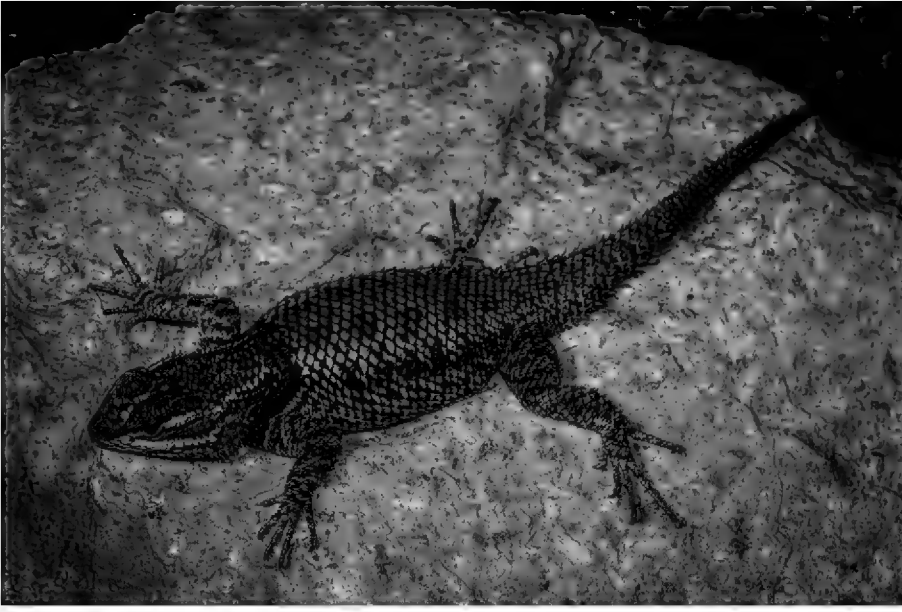
Gatica-Colima et al. (2014c) reported the first member of the tick family Argasidae associated with the Prairie Rattlesnake (*Crotalus viridis*) from Janos (LMN

subprovince), Chihuahua, as well as the second record of an argasid tick on a snake from Mexico.

On a cattle ranch located in Jeff Davis and Presidio counties, Texas, Christensen et al. (2020) tracked the development of a previously undescribed disease, Carapacial Shell Disease Process (CSDP), in the Yellow Mud Turtle (*Kinosternon flavescens*) over a 13-year period. By using permanent artificial water sources, a filamentous alga (*Arnoldiella chelonum*) invaded and damaged the non-living portion of the shell of this species. Subsequently, the authors examined 475 dry and fluid preserved *K. flavescens* that were collected in 121 counties in Texas, and 73 (60.3%) demonstrated the presence of this disease. Records of this disease have been found in the five Texas counties that border Chihuahua. In Chihuahua, this turtle is distributed in the SPN and LSV subprovinces.



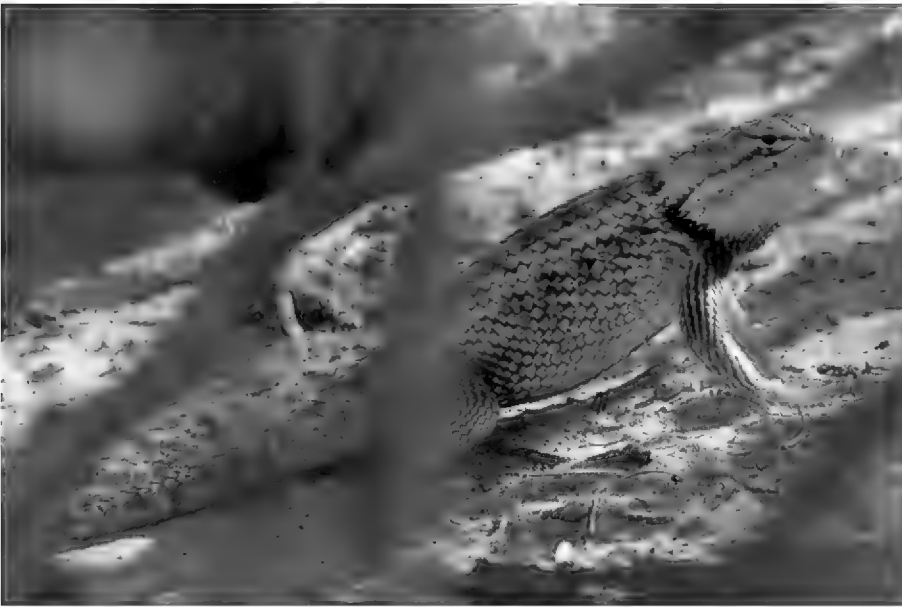
Fig. 25. At least 10 blood sucking mosquitoes (Diptera) are seen on the body of a Black-tailed Rattlesnake (*Crotalus molossus*) in the municipality of Guerrero, Chihuahua. *Photo by Sara G. Sáenz-González.*



No. 29. *Sceloporus jarrovii* Cope, 1875. Yarrow's Spiny Lizard is distributed from "southeastern Arizona and southwestern New Mexico through the Sierra Madre Occidental of Chihuahua, Sonora, Sinaloa and Durango southward to southern Zacatecas and Aguascalientes" (Heimes 2022: 327). This lizard was photographed on a rock in pine forest at Monterde, in the municipality of Guazapares, Chihuahua. Wilson et al. (2013a) determined its EVS as 11, placing it in the middle portion of the medium vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 30. *Sceloporus lemosespinali* Lara-Góngora, 2004. The Western Graphic Lizard "occurs in the Sierra Madre Occidental and associated mountain ranges of Chihuahua and Sonora, ranging southward into northern Durango and Sinaloa" (Heimes 2022: 128). This individual was photographed on dry bark in a pine forest near Creel, in the municipality of Bocoyna, Chihuahua. Wilson et al. (2013a) calculated its EVS as 16, placing it in the middle portion of the high vulnerability category. The IUCN evaluated its conservation status as Data Deficient, and this species is not listed by SEMARNAT. *Photo by Antonio Esaú Valdenegro-Brito.*



No. 31. *Sceloporus magister* Hallowell, 1854. The Desert Spiny Lizard "occurs in the Sonoran Desert from southwestern Arizona through western Sonora, including Isla Tiburón in the Gulf of California, southward to northwestern Sinaloa" (Heimes 2022: 338). This adult individual, which lacks the tip of its tail, was photographed in a sand dune under Sand Sagebrush (*Artemisa filifolia*), near Ojo de la Punta in the Samalayuca Dune Fields, Juárez, Chihuahua. Wilson et al. (2013a) calculated its EVS as 9, placing it at the upper limit of the low vulnerability category. The IUCN determined its conservation status as Least Concern, but this species is not listed by SEMARNAT. *Photo by Jesús M. Martínez-Calderas.*



No. 32. *Sceloporus merriami* Stejneger, 1904. The Canyon Lizard "ranges widely from the Big Bend region of southwestern Texas southward through eastern Chihuahua and Coahuila to extreme northeastern Durango and west-central Nuevo León, but its distribution is highly discontinuous" (Heimes 2022: 343). This individual was photographed in a rocky outcrop at Angulo canyon, a Natural Protected Area (Cañón de Santa Elena) in the municipality of Manuel Benavides, Chihuahua. Wilson et al. (2013a) determined its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Daisy Marina Cuevas Ortalejo.*



Fig. 26. Road-killed snakes often are seen on the roads in Chihuahua. Here, a dead adult Prairie Rattlesnake (*Crotalus viridis*) was found in a Type C road in Janos, Chihuahua. *Photo by Ana Gatica-Colima.*



Fig. 27. Chihuahua is one of Mexico's leading states for mining activities, and the tailings often contaminate water resources. Pictured here is an abandoned mine at Bustillos, in the municipality of Aquiles Serdán, Chihuahua. *Photo by Ana Gatica-Colima.*

On and off-road activities (Fig. 26)

The number and species of reptiles killed by vehicles on roads is likely underestimated, not only because of their relatively small size and removal by scavengers, but also because they are often flattened beyond recognition. Traffic on roads also causes noise pollution, interfering with the vocal communication of certain animals. Frogs living along noisy roads are known to increase the amplitude or pitch of their calls. Roads also can rapidly cause genetic effects, thereby raising conservation concerns about rare and threatened species, as documented in a review by Dean et al. (2019). Lazcano et al. (2009a,b, 2019) documented other impacts of roads on snake populations.

The Ramsar area along the Río San Pedro-Meoqui, Chihuahua (No. 2047) is subject to constant anthropogenic disturbances, including extreme sports practices with Off-Road Vehicles (ORV) as recorded

in 2009 (see Loredó-Varela and Hernández-Escudero 2021).

The establishment of factories that manufacture recreational vehicles and power sports engines for aquatic, snow, and all-terrain vehicles keeps increasing in Juárez, Chihuahua. Off-Road Vehicles (ORV) and racing activities have increased in northern Chihuahua, sometimes in natural landscapes that have not been assigned for recreational use, and they can potentially affect the flora and fauna of this region. A critical evaluation of this situation is required to document the impact caused by these activities.

In a conservation study conducted by Hans-Werner et al. (2017), the authors found reduced gene flow in a population of an important reptilian predator, the Western Diamond-backed Rattlesnake (*Crotalus atrox*), along Interstate 10 in the Sonoran Desert of southern Arizona. To reverse the trend, the authors recommended designing eco-passages to re-establish population connectivity.

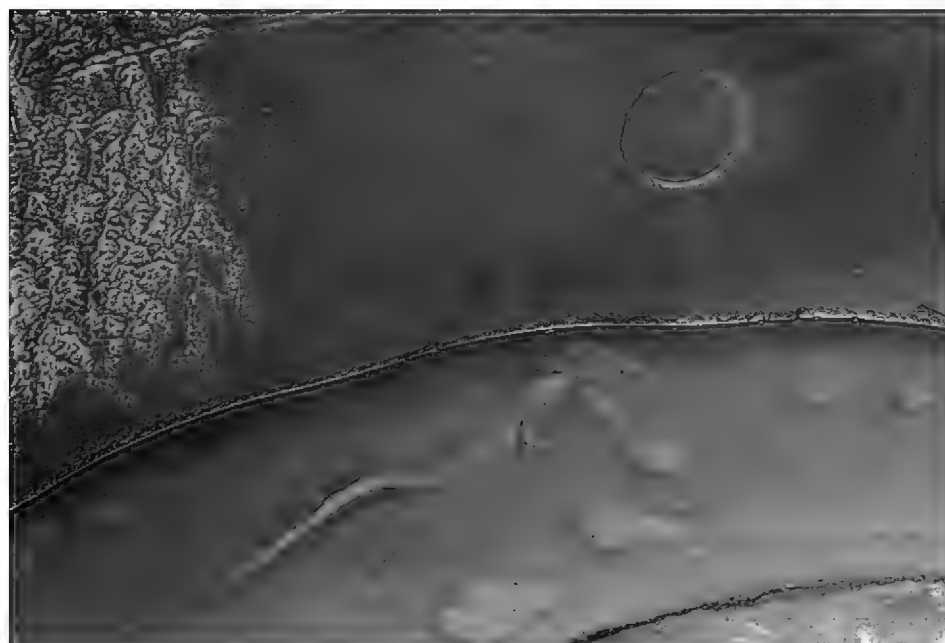


Fig. 28. Discarded tires sometimes are a threat to reptiles. Here, a Whiptail Lizard (*Aspidoscelis* sp.) was found dead inside of a discarded tractor tire in Ascensión, Chihuahua. Photo by Sandra I. Ramos Guerra.



Fig. 29. Recent observations of individual rattlesnakes and their venom indicate that they are being used in spiritual drinks. Here, a rattlesnake has been added to a bottle of Sotol, an alcoholic drink available in the northern states of Mexico. Photo by Ana Gatica-Colima.

Mining (Fig. 27)

In 2020, the primary minerals produced by municipalities in the state of Chihuahua were dolomite in the city of Chihuahua, silver in Guadalupe y Calvo and Chínipas, iron in Camargo, gypsum in Guadalupe, kaolin in the city of Chihuahua, zinc and lead in Santa Barbara, copper in Urique, and gold in Ocampo and Madera (INEGI 2022).

Sasaki et al. (2015) noted that structural alterations in terrestrial habitats, and concomitant changes in the availability of resources and microclimate, have had stronger effects on amphibian and reptile communities than metal pollution caused by century-long smelting operations. Other environmental impacts of mining are discussed by Gutiérrez-Ruiz et al. (2007), Gavilán García et al. (2017), and Guzmán-Martínez et al. (2023).

Mayani-Parás et al. (2019) produced ecological niche models for 179 amphibian and reptile species endemic to Mexico and examined the impact of habitat loss and mining activities. The lowest and highest values for the percentage of distribution loss for some endemic herpetofauna in Chihuahua are as follows: for amphibians, *Ambystoma rosaceum* (10.09, 14.34) and *A. velasci* (75.11, 75.83); and for reptiles, *Crotalus basiliscus* (25.93, 29.63) and *Salvadora bairdi* (60.54, 61.80).

Solid waste pollution (Fig. 28)

Gatica-Colima et al. (2016) found a dead adult Texas Horned Lizard (*Phrynosoma cornutum*) entangled in a discarded plastic crate in an illegal dump site in Ciudad Juárez, Chihuahua. Later, a live one was found inside a tire, and nine others were found dead in other tires. A subsequent survey demonstrated a relationship between the mortality of *P. cornutum* and discarded tires, and the authors commented that it could lead to local population extinction.

The Ramsar site of Río San Pedro-Meoqui, Chihuahua (No. 2047) is an area that suffers constant anthropogenic disturbances, such as garbage disposal recorded in 2018 (see Loredó-Varela and Hernández-Escudero 2021). Recently, Dong-Min and Ding-Qi (2022) reviewed microplastics and their effects on amphibians and reptiles, focusing on the toxicity of bisphenol A (BPA) to tadpoles, the toxic effects caused by direct contact or ingestion, and the detection of microplastics in the natural environment of amphibians. In addition, reptiles can become entangled in plastics and might ingest large pieces of this material.

Consumption of amphibians and reptiles (Fig. 29)

Gatica-Colima and Jiménez-Castro (2009) studied the uses of rattlesnakes by local people, and reported that

in the Chihuahuan Desert, and primarily in Chihuahua, people consume rattlesnakes as food and for medicinal purposes.

An alcoholic drink called “sotol” is produced in the state of Chihuahua, particularly in the desert and mountainous municipalities that gave rise to the product’s name and the historical tradition for its process in artisan factories (Diario Oficial de la Federación 2002). Recently, the addition of rattlesnakes (whole individuals or their venom) to Sotol has been increasing (Fig. 23). An evaluation is needed to confirm the attributes of this drink with rattlesnake products, especially when using endangered species.

Agriculture and livestock grazing (Figs. 30–34)

According to the Secretariat of the Convention of Biological Diversity SCBD (2008), the homogenization of farming landscapes with the elimination of natural areas, including hedgerows, woodlots and wetlands, to achieve larger scale production units for large-scale mechanized production also has led to declines in biodiversity and ecological services. This is only one way that agriculture can reduce biodiversity.

From 2002–2014 there was a tendency for extending the anthropic origin for annual and perennial agriculture in Mexico. Extensive zones of negative changes in the desert regions of Sonora and Chihuahua have been noted, with reductions in the xerophytic shrubs (INEGI 2021c).

Pérez-Espejo (2008) commented on the difficulty of quantifying biodiversity loss due to cattle. An estimate of the role of livestock among the threats and losses of biodiversity was based on the emission of greenhouse gases, soil erosion, and water pollution.

The PMARP (2012) identified a total of 10 major problems or necessities for a regional alliance for conserving grasslands of the Chihuahuan Desert in the states of Zacatecas and Chihuahua, one of which is the animal load adjustment (i.e., the amount of livestock that grassland ideally can support).

The use of chemicals in agricultural and livestock areas also are a constant threat to wildlife, particularly for groups such as amphibians due to the continuous interchange of gases through their skin (Siliceo-Cantero 2021).

Fear and/or confusion

Saucedo-Sánchez de Tagle (2007) attempted to bring Rarámuri students closer to classifying fauna based on their conception of the universe, where animals and diverse beings are grouped around the celestial region of the Tarahumaran cosmos. In this study, the author identified the chameleon (*wilókare*) as capable of curing certain diseases by placing it on the part of the body with an ailment, and allowing the reptile to “suck the bad blood.” Subsequently, Alonso-Castro (2014) documented the use of ethnozoology for medicinal purposes in Mexican Traditional Medicine (MTM), and mentioned *Anolis carolinensis* as the “chameleon” that was used by the Tarahumaras. Caution must be taken when referring to a given species in MTM, as the information provided

usually is based on common names. According to Conant and Collins (1991), the Green Anole (*Anolis carolinensis*) was reported as an isolated record from Tamaulipas, Mexico, where presumably it was introduced.

According to a survey by Gatica and Jiménez-Castro (2009), some people indicated they might kill rattlesnakes 48.93% ($n = 23$), while another 31.91% ($n = 15$) may not. About one-half of the people that kill them do so because of fear or precaution ($n = 19$), whereas, others consume them (10.52%), and there is also a combination of fear and consumption 31.57% ($n = 12$).

Fear is the principal enemy of conservation, and thus the need for more education.

Miscellaneous threats

The Priority Terrestrial Region (RTP) project is circumscribed in the Priority Regions Program for the Conservation of Biodiversity of the National Commission for the Knowledge and Use of Biodiversity (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad—CONABIO), and this commission identifies ecological areas with physical and biotic characteristics that favor biodiversity. Twenty-one (13.81%) of the 152 national RTP’s are recognized in Chihuahua. In Appendix 1, we compiled the principal environmental problems in the state RTPs (Arriaga et al. 2000) that are associated with the physiographic provinces in Chihuahua.

Conservation Status

In this study, we used the three systems of conservation assessment employed in all the other entries in the MCS (see above), i.e., the systems of SEMARNAT (2010), the IUCN Red List (<http://www.iucnredlist.org>), and the EVS (Wilson et al. 2013a,b). The assessments from these three systems were updated as necessary to reflect the most current information.

The SEMARNAT System

Torres-Hernández et al. (2021: 117) indicated that “the SEMARNAT system for assessing conservation status was developed and implemented by the Secretaría de Medio Ambiente y Recursos Naturales of the federal government of Mexico (SEMARNAT 2010, 2019).” We provide the status ratings for the native herpetofaunal species in Chihuahua in Table 7, and summarize the data in Table 11. Three evaluation categories are established in the SEMARNAT system: Endangered (P), Threatened (A), and Under Special Protection (Pr) for species considered under threat. In addition, we allocated the species that were not assessed to a “No Status” (NS) category (Tables 7 and 11).

As with all the previous MCS studies, we found that the SEMARNAT system, by design, has been applied to relatively few of the native members of the herpetofauna (Table 11). In the case of Chihuahua, only 68 of the 183 native species (37.2%) have been evaluated, with three species allocated to the Endangered (P) category, 23 to the Threatened (A) category, and 42 to the Special Protection



Fig. 30. The use of different agricultural products has been increasing in various areas of Chihuahua, such as extensive fields of corn in the arid lands of the state. *Photo by Ana Gatica-Colima.*



Fig. 31. Extensive cotton fields have been increasing in Mennonite communities in several municipalities of Chihuahua, thereby transforming the natural habitats. *Photo by Ana Gatica-Colima.*



Fig. 32. In recent years, the planting of pecans and walnuts has increased in the state of Chihuahua. Pictured here is a field of pecans in Meoqui. *Photo by Ana Gatica-Colima.*



Fig. 33. Traditionally, Chihuahua has been a cattle-producing state. Here, a herd of cows roams free in the municipality of Casas Grandes, Chihuahua. *Photo by Ana Gatica-Colima.*



Fig. 34. Cattle guards or grids are structures that prevent livestock from crossing enclosed properties. Recent observations reveal, however, that cattle guards also serve as traps for reptiles and other vertebrates. Here, we encountered and rescued four Texas Horned Lizards (*Phrynosoma cornutum*) from under the shade of a grid. *Photo by Ana Gatica-Colima.*



No. 33. *Sceloporus poinsettii* Baird and Girard, 1852. The distribution of the Crevice Spiny Lizard “extends from central New Mexico and central Texas, in the United States, southward in Mexico to northern Jalisco, west of the Sierra Madre Occidental; in Chihuahua, it ranges westward to the Pacific slopes of the Sierra Madre Occidental and eastward to the western slopes of the Sierra Madre Oriental in the states of Nuevo León and northern and north-central San Luis Potosí” (Lemos-Espinal and Dixon 2013: 133–134). This individual was photographed on a rock with lichens at Sierra El Capulín, in Ascensión, Chihuahua. Wilson et al. (2013a) assessed its EVS as 12, placing it in the middle of the medium vulnerability category. The IUCN judged the conservation status of this lizard as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eduardo Francisco Macias-Rodríguez.*



No. 34. *Sceloporus virgatus* Smith, 1938. The Striped Plateau Lizard “occurs in the Sierra Madre Occidental and associated mountains of western Chihuahua and eastern Sonora, possibly ranging southward into the northern part of Durango” (Heimes 2022: 401). This individual was photographed on dry pine leaves in pine forest at Monterde, in the municipality of Guazapares, Chihuahua. Wilson et al. (2013a) determined its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 35. *Urosaurus ornatus* (Baird and Girard, 1852). The Ornate Tree Lizard is distributed from “extreme southwestern Wyoming and central Texas southward to western and northern Mexico. In Mexico, it occurs on the Pacific side from the Rio Colorado valley of northeastern Baja California through all of Sonora (including Isla Tiburón and some smaller islands in the Sea of Cortés) and Sinaloa southward to Nayarit” (Heimes 2022: 421). This individual was photographed as it was climbing a mesquite tree near El Paradero, in the municipality of Ojinaga, Chihuahua. Wilson et al. (2013a) calculated its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN determined its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Cesar Daniel Muñoz-Rivas.*



No. 36. *Uta stansburiana* (Baird and Girard, 1852). The Common Side-blotched Lizard is “widely distributed in desert regions of the western United States and northern Mexico, ranging from central Washington southeast to western Texas, and south to Baja California, Sonora and the Mexican Plateau...On the Mexican Plateau, the range extends from northern and eastern Chihuahua southeast through western and southern Coahuila to adjacent west-central Nuevo León, and south to northeastern Durango and extreme northern Zacatecas...” (Heimes 2022: 429). This adult male was photographed in sandy soil near San Gerónimo ranch, north of Sierra Samalayuca, Juárez, Chihuahua. Wilson et al. (2013a) calculated its EVS as 7, placing it in the middle portion of the low vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ana B. Gatica-Colima.*

The herpetofauna of Chihuahua, Mexico

Table 11. SEMARNAT categorizations for the herpetofaunal species in Chihuahua, Mexico, arranged by families. Non-native species are excluded.

Family	Number of species	SEMARNAT categorization			
		Endangered (P)	Threatened (A)	Special Protection (Pr)	No Status (NS)
Bufonidae	11	—	—	1	10
Craugastoridae	2	—	—	1	1
Eleutherodactylidae	2	—	—	1	1
Hylidae	4	—	—	—	4
Microhylidae	3	—	—	1	2
Phyllomedusidae	1	—	—	—	1
Ranidae	8	—	1	3	4
Scaphiopodidae	3	—	—	—	3
Subtotal	34	—	1	7	26
Ambystomatidae	4	—	—	2	2
Plethodontidae	1	—	—	—	1
Subtotal	5	—	—	2	3
Amphibia total	39	—	1	9	29
Anguidae	5	—	—	3	2
Anolidae	1	—	—	—	1
Crotaphytidae	2	—	1	1	—
Eublepharidae	1	—	—	1	—
Helodermatidae	2	—	2	—	—
Iguanidae	1	—	—	—	1
Phrynosomatidae	23	1	3	—	19
Phyllodactylidae	1	—	—	—	1
Scincidae	7	—	—	3	4
Teiidae	9	—	—	2	7
Subtotal	52	1	6	10	35
Boidae	1	—	—	—	1
Colubridae	40	—	7	3	30
Dipsadidae	10	—	—	4	6
Elapidae	2	—	1	1	—
Leptotyphlopidae	4	—	—	—	4
Natricidae	11	—	6	1	4
Viperidae	11	—	—	9	2
Subtotal	79	—	14	18	47
Emydidae	4	—	1	2	1
Geoemydidae	1	—	1	—	—
Kinosternidae	5	1	—	2	2
Testudinidae	2	1	—	—	1
Trionychidae	1	—	—	1	—
Subtotal	13	2	2	5	4
Reptiles total	144	3	22	33	86
Sum total	183	3	23	42	115

(Pr) category. Most species (115, or 62.8%) remain unassessed.

Of the three species allocated to the Endangered category, one is a country endemic lizard (*Uma paraphygas*), one is a non-endemic turtle (*Kinosternon sonoriense*), and one is a country endemic turtle (*Gopherus flavomarginatus*). Of the 23 species placed in the Threatened (A) category, one is a non-endemic frog (*Lithobates chiricahuensis*), four are non-endemic lizards (*Crotaphytus collaris*, *Heloderma suspectum*, *Cophosaurus texanus*, and *Uta stansburiana*), two are country endemic lizards (*Heloderma horridum* and *Phrynosoma orbiculare*), 11 are non-endemic snakes (*Lampropeltis alterna*, *Masticophis flagellum*, *M. mentovarius*, *Tantilla atriceps*, *Trimorphodon wilkinsonii*, *Micruroides euryxanthus*, *Nerodia erythrogaster*, *Thamnophis cyrtopsis*, *T. elegans*, *T. eques*, and *T. marciamus*), three are country endemic snakes (*Leptophis diplotropis*, *Pituophis deppei*, and *Thamnophis melanogaster*), and two are non-endemic turtles (*Chrysemys picta* and *Rhinoclemmys pulcherrima*).

Forty-two species are placed in the Special Protection (Pr) category, including seven anurans, two salamanders, 10 lizards, 18 snakes, and five turtles (Table 11).

Given that only slightly more than one-third of the native species of amphibians and reptiles have been assessed by the SEMARNAT system, this system is of little use in assessing the conservation status of the herpetofauna of Chihuahua, although the system does deal with the threatened species identified by SEMARNAT.

The IUCN System

The IUCN system of conservation assessment has a global reach and is the most widely-used system in Mexican herpetology. In our estimation, however, this system has several drawbacks, as identified in earlier entries in the MCS. Nonetheless, we would be remiss if we did not examine its application to the herpetofauna of Chihuahua.

The IUCN system of conservation assessment encompasses six categories, including three so-called “threat” categories of Critically Endangered (CR),

Endangered (EN), and Vulnerable (VU); two categories of so-called “lesser risk,” i.e., Near Threatened (NT) and Least Concern (LC); and one category called Data Deficient (DD) for species for which too little information exists to allow for assignment to another category. Finally, we use a category of Not Evaluated (NE) for species that have not been assessed using the IUCN system. Two additional categories exist for species considered to be either Extinct (EX) or Extinct in the Wild (EW), but these categories seldom apply to herpetofaunal species.

We provide the data on the IUCN categorizations in Table 7, and a summary of the data in Table 12. The data in Table 12 show that only seven species are allocated to the “threat categories” of CR, EN, and VU. The single CR species is the country endemic *Gopherus flavomarginatus**. The single EN species is the country endemic *Thamnophis melanogaster**. The five VU species are the anurans *Lithobates chiricahuensis* and *L. tarahumarae*, the salamander *Isthmura sierraoccidentalis**, and the turtles *Trachemys gaigeae* and *Gopherus evgoodei**. Two of these VU species are country endemics (indicated by the asterisks). Of the 148 species of “lesser risk,” most (142, or 95.9%) are allocated to the Least Concern (LC) category. An additional 11 species are assigned to the Data Deficient (DD) category, which leaves 17 species unassessed, so we assigned them to the Not Evaluated (NE) category.

Of the 148 “lesser risk” species, 39 are country endemics (26.4%), and the remainder are non-endemics (Table 7). The 11 Data Deficient (DD) species are as follows: *Lithobates lemosespinali**, *Ambystoma silvense**, *Barisia levicollis**, *Sceloporus lemosespinali**, *Plestiodon multilineatus***, *P. parviauriculatus**, *Mastigodryas cliftoni**, *Sympholis lippiens**, *Tropidodipsas repleta**, *Terrapene nelsoni**, and *Kinosternon durangoense**. All 11 species are country endemics, except for *Plestiodon multilineatus*, which is the single Chihuahua state endemic species. The species not evaluated by the IUCN include one anuran, six lizards, nine snakes, and one turtle. Of these 17 species, eight (47.1%) are country endemics (Table 7).

The 142 species allocated to the Least Concern (LC) category constitute 77.6% of the 183 native members of the Chihuahua herpetofauna. Of these 142 species, 38 (26.8%) are country endemics, and the remainder are non-endemics. As found in the previous MCS entries, most of the native species in Chihuahua (slightly more than three-quarters) have been placed in the Least Concern (LC) category. We consider the LC category to be overused by the IUCN, which creates a false impression that, from a conservation perspective, the native herpetofauna generally is in better shape than reported in the MCS studies. Since this pattern consistently has been observed in these studies, we apply the EVS system below.

The EVS System

The Environmental Vulnerability Score (EVS) system of conservation assessment originally was developed for use with the herpetofauna of Honduras (Wilson and McCranie 2004), because the herpetofauna was not sufficiently well understood to apply the criteria used

in the IUCN system at that time. Subsequently, the EVS system has been applied to the entire Mexican and Central American herpetofauna (Wilson et al. 2013a,b; Johnson et al. 2015a), as well as in all the previous MCS entries (see above). In addition, this system is being used more frequently in other conservation studies on the herpetofauna of Mexico, including those by Julio Lemos-Espinal and his co-authors.

In this entry, we determined the EVS values for the 183 native members of the herpetofauna of Chihuahua. We present these values in Table 7 and summarize them in Table 13. The EVS values range from 3 to 19, one less than the total theoretical range of values (3–20). The most frequent values (i.e., those associated with 10 or more species) are: 8 (11 species), 10 (16 species), 11 (25 species), 12 (17 species), 13 (20 species), 14 (29 species), and 15 (17 species). Notably, we collectively applied these seven values to 135 (73.8%) of the 183 native species in Chihuahua. The lowest score of 3 was calculated for only three anuran species (*Rhinella horribilis*, *Smilisca baudinii*, and *Scaphiopus couchii*). The highest value of 19 was applied to only two species, one a lizard (*Ctenosaura macrolopha**) and a turtle (*Gopherus flavomarginatus**).

As in all other MCS studies, we grouped the EVS values into three categories of low (3–9), medium (10–13), and high (14–19) vulnerability. Based on this categorization, the resulting EVS values for the native members of the Chihuahua herpetofauna increase from low vulnerability (41 species) to medium vulnerability (78 species), and then decrease with high vulnerability (64 species). This pattern of an increase from low to medium and a decrease from medium to high is similar to the patterns in many of the MCS studies (e.g., Barragán-Vázquez et al. 2022 and Leyte-Manrique et al. 2022).

To demonstrate how the IUCN ratings relate to those for the EVS, we compared the categorizations for these two systems in Table 14. Only seven (10.9%) of the 64 high vulnerability species are allocated to the three IUCN “threat categories” (CR, EN, and VU). At the other extreme, 41 of the low vulnerability species (by EVS) account for only 28.9% of the 142 LC species (by IUCN). Consequently, as seen in many of the other MCS studies, the conservation evaluations provided by the IUCN and EVS systems do not correlate well with one another.

As shown in previous MCS studies, the primary reason for the poor correspondence between the IUCN and EVS systems of conservation evaluation is the sizable number of species placed in the IUCN’s DD, NE, and LC categories. In the case of the Chihuahuan herpetofauna, these categorizations involve 170 (92.9%) of the 183 native species. Of these 170 species, 11 are allocated to the DD category (Table 15). One of these species is an anuran, one is a salamander, four are lizards, three are snakes, and two are turtles. All 11 are endemic species, including 10 country endemics and one state endemic; their EVS values range from 14 to 18, all high vulnerability values. Unless the conservation status of these species is reassessed as additional information accumulates, then for all intents

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Table 12. IUCN Red List categorizations for the herpetofaunal families in Chihuahua, Mexico. Non-native species are excluded. The shaded columns to the left are the “threat categories,” and those to the right are the categories for which insufficient information on conservation status is available to place the taxa in another IUCN category, or they have not been evaluated.

Family	Number of species	IUCN Red List categorization						
		Critically Endangered	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated
Bufonidae	11	—	—	—	—	10	—	1
Craugastoridae	2	—	—	—	—	2	—	—
Eleutherodactylidae	2	—	—	—	—	2	—	—
Hylidae	4	—	—	—	—	4	—	—
Microhylidae	3	—	—	—	—	3	—	—
Phyllomedusidae	1	—	—	—	—	1	—	—
Ranidae	8	—	—	2	—	5	1	—
Scaphiopodidae	3	—	—	—	—	3	—	—
Subtotal	34	—	—	2	—	30	1	1
Ambystomatidae	4	—	—	—	—	3	1	—
Plethodontidae	1	—	—	1	—	—	—	—
Subtotal	5	—	—	1	—	3	1	—
Amphibia total	39	—	—	3	—	33	2	1
Anguidae	5	—	—	—	—	4	1	—
Anolidae	1	—	—	—	—	1	—	—
Crotaphytidae	2	—	—	—	—	2	—	—
Eublepharidae	1	—	—	—	—	1	—	—
Helodermatidae	2	—	—	—	1	1	—	—
Iguanidae	1	—	—	—	—	1	—	—
Phrynosomatidae	23	—	—	—	1	18	1	3
Phyllodactylidae	1	—	—	—	—	—	—	1
Scincidae	7	—	—	—	—	4	2	1
Teiidae	9	—	—	—	—	8	—	1
Subtotal	52	—	—	—	2	40	4	6
Boidae	1	—	—	—	—	—	—	1
Colubridae	40	—	—	—	1	34	2	3
Dipsadidae	10	—	—	—	—	9	1	—
Elapidae	2	—	—	—	—	2	—	—
Leptotyphlopidae	4	—	—	—	—	2	—	2
Natricidae	11	—	1	—	—	8	—	2
Viperidae	11	—	—	—	1	9	—	1
Subtotal	79	—	1	—	2	64	3	9
Emydidae	4	—	—	1	1	1	1	—
Geoemydidae	1	—	—	—	—	—	—	1
Kinosternidae	5	—	—	—	1	3	1	—
Testudinidae	2	1	—	1	—	—	—	—
Trionychidae	1	—	—	—	—	1	—	—
Subtotal	13	1	—	2	2	5	2	1
Reptiles total	144	1	1	2	6	109	9	16
Sum total	183	1	1	5	6	142	11	17
Category total	183	7			148		28	

and purposes these species are ignored. In our opinion, the four species with an EVS of 14 (*Lithobates lemosespinali**, *Ambystoma silvense**, *Mastigodryas cliftoni**, and *Sympholis lippiens**) should be placed in the IUCN VU category, the five species with an EVS of 15 or 16 (*Barisia levicollis**, *Sceloporus lemosespinali**, *Plestiodon multilineatus*** [the single Chihuahua state endemic], *P. parviauriculatus**, and *Kinosternon durangoense**) should be allocated to the EN category, and the two species with an EVS of 17 or 18 (*Tropidodipsas repleta** and *Terrapene nelsoni**) should be relegated to the CR category.

Seventeen species remain unevaluated by the IUCN (Table 16). One species is an anuran, six are lizards, nine are snakes, and one is a turtle. Eight species are country endemics, and the remaining nine are non-endemics. Their EVS values range from 3 to 16, with three in the low vulnerability category, six in the medium vulnerability category, and eight in the

high vulnerability category. The three species with an EVS of 3 to 8 should be placed in the LC category, the six with EVS of 11 to 13 in the NT category, the four species with EVS of 14 in the VU category, and the four species with an EVS of 15 or 16 in the EN category.

The IUCN placed the largest number of species that comprise the Chihuahuan herpetofauna in the LC category (Table 17). The 142 LC species includes 30 anurans, three salamanders, 40 lizards, 64 snakes, and five turtles. Thirty-four (23.9%) of these 142 species are country endemics and 108 (76.1%) are non-endemics. Their EVS values range from 3 to 19, with 37 falling within the low vulnerability category, 69 in the medium vulnerability category, and 36 in the high vulnerability category. Fifty-three species have an EVS ranging from 3 to 10 and can be placed in the LC category, the 54 with an EVS from 11 to 13 can be allocated to the NT category, the 21 with an EVS of 14

Table 13. Environmental Vulnerability Scores (EVS) for the herpetofaunal species in Chihuahua, Mexico, arranged by family. The shaded area on the left encompasses low vulnerability scores, and the one on the right includes the high vulnerability scores. Non-native species are excluded.

Family	Number of species	Environmental Vulnerability Score																
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Bufonidae	11	1	—	1	—	1	—	1	1	2	2	1	1	—	—	—	—	—
Craugastoridae	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—
Eleutherodactylidae	2	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—
Hylidae	4	1	—	—	—	1	—	1	—	1	—	—	—	—	—	—	—	—
Microhylidae	3	—	1	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Phyllomedusidae	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
Ranidae	8	—	—	—	—	1	1	1	—	1	2	1	1	—	—	—	—	—
Scaphiropodidae	3	1	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—
Subtotal	34	3	1	1	1	3	3	4	2	5	4	3	2	1	—	1	—	—
Ambystomatidae	4	—	—	—	—	—	—	—	2	—	—	—	2	—	—	—	—	—
Plethodontidae	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Subtotal	5	—	—	—	—	—	—	—	2	—	—	—	2	—	—	1	—	—
Amphibia total	39	3	1	1	1	3	3	4	4	5	4	3	4	1	—	2	—	—
Anguidae	5	—	—	—	—	—	—	—	1	—	—	1	1	2	—	—	—	—
Anolidae	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
Crotaphytidae	2	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
Eublepharidae	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Helodermatidae	2	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—
Iguanidae	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Phrynosomatidae	23	—	—	—	—	1	—	1	3	3	4	5	2	1	2	1	—	—
Phyllodactylidae	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Scincidae	7	—	—	—	—	—	—	—	—	1	2	1	1	1	1	—	—	—
Teiidae	9	—	—	—	—	—	—	1	—	1	—	1	4	2	—	—	—	—
Subtotal	52	—	—	—	—	1	—	2	4	6	6	11	9	7	4	1	—	1
Boidae	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Colubridae	40	—	—	2	4	1	2	2	5	8	2	2	8	3	1	—	—	—
Dipsadidae	10	—	1	—	2	—	1	—	1	1	1	1	1	—	—	1	—	—
Elapidae	2	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
Leptotyphlopidae	4	—	—	—	—	—	2	—	—	—	—	1	1	—	—	—	—	—
Natricidae	11	—	—	—	—	1	1	—	1	2	1	—	2	1	2	—	—	—
Viperidae	11	—	—	—	—	—	1	1	—	2	2	2	1	1	1	—	—	—
Subtotal	79	—	1	2	6	2	7	3	7	13	6	6	14	7	4	1	—	—
Emydidae	4	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	2	—
Geoemydidae	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Kinosternidae	5	—	—	—	—	—	—	—	1	1	1	—	1	—	1	—	—	—
Testudinidae	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Trionychidae	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Subtotal	13	—	—	—	—	—	1	—	1	1	1	—	2	2	1	—	3	1
Reptiles total	144	—	1	2	6	3	8	5	12	20	13	17	25	16	9	2	3	2
Sum total	183	3	2	3	7	6	11	9	16	25	17	20	29	17	9	4	3	2
Category total	183	41					78					64						

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No. 37. *Plestiodon bilineatus* (Tanner, 1958). The Two-lined Short-nosed Skink “occurs in the Sierra Madre Occidental from southern Chihuahua to northern Jalisco” (Lemos-Espinal et al. 2019: 150–151). This individual was photographed on dry leaves in pine forest at Monterde, in the municipality of Guazapares, Chihuahua. Wilson et al. (2013a) determined its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. Photo by Eric Centenero-Alcalá.



No. 38. *Aspidoscelis exsanguis* (Lowe, 1956). The Chihuahuan Spotted Whiptail occupies “Most of New Mexico, western Texas, northeastern Sonora and most of Chihuahua east of the Sierra Madre Occidental” (Lemos-Espinal et al. 2013: 84). This adult individual was photographed on a small hill with oak trees, near Cueva de las Monas, municipality of Chihuahua, Chihuahua. Wilson et al. (2013a) determined its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN calculated its conservation status as Least Concern, and this species is not listed by SEMARNAT. Photo by Ana B. Gatica-Colima.



No. 39. *Aspidoscelis marmoratus* (Baird and Girard, 1852). The Marbled Whiptail occupies “Parts of New Mexico and western Texas south through northern and eastern Chihuahua to northeastern Durango, southern Coahuila, and western and southern Nuevo León” (Lemos-Espinal et al. 2018b: 137). This individual was photographed in sandy soil containing sand dune vegetation near the microwave tower station at Ascensión, Chihuahua. Wilson et al. (2013a) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN has not judged its conservation status, and this species is not listed by SEMARNAT. Photo by Sandra I. Ramos-Guerra.



No. 40. *Aspidoscelis tessellatus* (Say, in James, 1823). The Checkered Whiptail “is distributed from central New Mexico southward into western Texas and eastern Chihuahua...along the Río Bravo and Río Conchos” (Lemos-Espinal et al. 2007: 360–361). This individual was photographed in sandy soil on the bank of a dry creek at Ojinaga, Chihuahua. Wilson et al. (2013a) judged its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. Photo by Eduardo F. Macias-Rodríguez.

Table 14. Comparison of Environmental Vulnerability Scores (EVS) and IUCN categorizations for members of the herpetofauna of Chihuahua, Mexico. Non-native species are excluded. The shaded area at the top encompasses the low vulnerability category scores, and the one at the bottom includes the high vulnerability category scores.

EVS	IUCN categorization							Total
	Critically Endangered	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated	
3	—	—	—	—	2	—	1	3
4	—	—	—	—	2	—	—	2
5	—	—	—	—	3	—	—	3
6	—	—	—	—	7	—	—	7
7	—	—	—	—	6	—	—	6
8	—	—	1	—	8	—	2	11
9	—	—	—	—	9	—	—	9
10	—	—	—	—	16	—	—	16
11	—	—	1	1	21	—	2	25
12	—	—	—	—	16	—	1	17
13	—	—	—	—	17	—	3	20
14	—	—	—	1	20	4	4	29
15	—	1	—	2	11	2	1	17
16	—	—	—	1	2	3	3	9
17	—	—	1	1	1	1	—	4
18	—	—	2	—	—	1	—	3
19	1	—	—	—	1	—	—	2
Total	1	1	5	6	142	11	17	183

Table 15. Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Chihuahua, Mexico that are allocated to the IUCN Data Deficient category. * = country endemic. ** = Chihuahua endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic distribution	Ecological distribution	Reproductive mode/Degree of persecution	Total score
<i>Lithobates lemosespinali</i> *	5	8	1	14
<i>Ambystoma silvense</i> *	5	8	1	14
<i>Barisia levicollis</i> *	5	7	3	15
<i>Sceloporus lemosespinali</i> *	5	8	3	16
<i>Plestiodon multilineatus</i> **	5	8	3	16
<i>Plestiodon parviauriculatus</i> *	5	7	3	15
<i>Mastigodryas cliftoni</i> *	5	6	3	14
<i>Sympholis lippiens</i> *	5	6	3	14
<i>Tropidodipsas repleta</i> *	5	8	4	17
<i>Terrapene nelsoni</i> *	5	7	6	18
<i>Kinosternon durangoense</i> *	5	8	3	16

Table 16. Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Chihuahua, Mexico that are currently not evaluated (NE) by the IUCN. Non-native taxa are excluded. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic distribution	Ecological distribution	Reproductive mode/Degree of persecution	Total score
<i>Rhinella horribilis</i>	1	1	1	3
<i>Holbrookia approximans</i> *	5	6	3	14
<i>Sceloporus albiventris</i> *	5	8	3	16
<i>Sceloporus cowlesi</i>	4	6	3	13
<i>Phyllodactylus saxatilis</i> *	5	8	3	16
<i>Plestiodon bilineatus</i> *	5	5	3	13
<i>Aspidoscelis marmorata</i>	4	7	3	14
<i>Boa sigma</i> *	5	4	6	15
<i>Oxybelis microphthalmus</i>	2	6	3	11
<i>Salvadora deserticola</i>	4	6	4	14
<i>Salvadora lineata</i>	2	5	4	11
<i>Rena dugesii</i> *	5	8	1	14
<i>Rena segrega</i>	2	5	1	8
<i>Thamnophis unilabialis</i> *	5	7	4	16
<i>Thamnophis validus</i> *	5	3	4	12
<i>Crotalus ornatus</i>	4	4	5	13
<i>Rhinoclemmys pulcherrima</i>	1	4	3	8

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Table 17. Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Chihuahua, Mexico that are assigned to the IUCN Least Concern (LC) category. Non-native taxa are excluded. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic distribution	Ecological distribution	Reproductive mode/ Degree of persecution	Total score
<i>Anaxyrus cognatus</i>	3	5	1	9
<i>Anaxyrus debilis</i>	1	5	1	7
<i>Anaxyrus mexicanus</i> *	5	7	1	13
<i>Anaxyrus punctatus</i>	1	3	1	5
<i>Anaxyrus speciosus</i>	4	7	1	12
<i>Anaxyrus woodhousii</i>	3	6	1	10
<i>Incilius alvarius</i>	4	6	1	11
<i>Incilius mazatlanensis</i> *	5	6	1	12
<i>Incilius mccoysi</i> *	5	8	1	14
<i>Incilius occidentalis</i> *	5	5	1	11
<i>Craugastor augusti</i>	2	2	4	8
<i>Craugastor tarahumaraensis</i> *	5	8	4	17
<i>Eleutherodactylus interorbitalis</i> *	5	6	4	15
<i>Eleutherodactylus marnockii</i>	3	4	4	11
<i>Dryophytes arenicolor</i>	2	4	1	7
<i>Dryophytes wrightorum</i>	2	6	1	9
<i>Smilisca baudinii</i>	1	1	1	3
<i>Tlalocohyla smithi</i> *	5	5	1	11
<i>Gastrophryne mazatlanensis</i>	2	5	1	8
<i>Gastrophryne olivacea</i>	3	5	1	9
<i>Hypopachus variolosus</i>	2	1	1	4
<i>Agalychnis dacnicolor</i> *	5	5	3	13
<i>Lithobates berlandieri</i>	4	2	1	7
<i>Lithobates cora</i> *	5	7	1	13
<i>Lithobates magnaocularis</i> *	5	6	1	12
<i>Lithobates pustulosus</i> *	5	3	1	9
<i>Lithobates yavapaiensis</i>	4	7	1	12
<i>Scaphiopus couchii</i>	1	1	1	3
<i>Spea bombifrons</i>	3	6	1	10
<i>Spea multiplicata</i>	1	4	1	6
<i>Ambystoma mavortium</i>	3	6	1	10
<i>Ambystoma rosaceum</i> *	5	8	1	14
<i>Ambystoma velasci</i> *	5	4	1	10
<i>Barisia ciliaris</i>	5	7	3	15
<i>Barisia imbricata</i>	5	6	3	14
<i>Elgaria kingii</i>	2	5	3	10
<i>Gerrhonotus infernalis</i>	5	5	3	13
<i>Norops nebulosus</i> *	5	5	3	13
<i>Crotaphytus collaris</i>	3	7	3	13
<i>Gambelia wislizenii</i>	3	7	3	13
<i>Coleonyx brevis</i>	4	6	4	14
<i>Heloderma horridum</i> *	2	4	5	11
<i>Ctenosaura macrolopha</i>	5	8	6	19
<i>Cophosaurus texanus</i>	4	7	3	14
<i>Holbrookia elegans</i>	4	6	3	13
<i>Holbrookia maculata</i>	1	6	3	10
<i>Phrynosoma cornutum</i>	1	7	3	11
<i>Phrynosoma hernandesi</i>	3	7	3	13
<i>Phrynosoma modestum</i>	4	5	3	12
<i>Phrynosoma orbiculare</i> *	5	4	3	12
<i>Sceloporus clarkii</i>	2	5	3	10
<i>Sceloporus jarrovii</i>	2	6	3	11
<i>Sceloporus magister</i>	1	5	3	9
<i>Sceloporus merriami</i>	4	6	3	13
<i>Sceloporus nelsoni</i> *	5	5	3	13
<i>Sceloporus poinsettii</i>	4	5	3	12
<i>Sceloporus slevini</i>	2	6	3	11
<i>Sceloporus virgatus</i>	4	8	3	15
<i>Urosaurus bicarinatus</i> *	5	4	3	12
<i>Urosaurus ornatus</i>	2	5	3	10
<i>Uta stansburiana</i>	3	1	3	7
<i>Plestiodon callicephalus</i>	2	7	3	12
<i>Plestiodon multivirgatus</i>	3	8	3	14
<i>Plestiodon obsoletus</i>	3	5	3	11
<i>Plestiodon tetragrammus</i>	4	5	3	12

Table 17 (continued). Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Chihuahua, Mexico that are assigned to the IUCN Least Concern (LC) category. Non-native taxa are excluded. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic distribution	Ecological distribution	Reproductive mode/ Degree of persecution	Total score
<i>Aspidoscelis exsanguis</i>	4	7	3	14
<i>Aspidoscelis gularis</i>	2	4	3	9
<i>Aspidoscelis inornata</i>	4	7	3	14
<i>Aspidoscelis neomexicanus</i>	4	8	3	15
<i>Aspidoscelis sonora</i>	4	6	3	13
<i>Aspidoscelis tessellata</i>	4	7	3	14
<i>Aspidoscelis uniparens</i>	4	8	3	15
<i>Arizona elegans</i>	1	1	3	5
<i>Bogertophis subocularis</i>	4	7	3	14
<i>Conopsis nasus</i> *	5	4	2	11
<i>Drymarchon melanurus</i>	1	1	4	6
<i>Drymarchon margaritiferus</i>	1	1	4	6
<i>Gyalopion canum</i>	4	3	2	9
<i>Gyalopion quadrangulare</i>	3	6	2	11
<i>Lampropeltis alterna</i>	4	7	3	14
<i>Lampropeltis knoblochi</i>	2	5	3	10
<i>Lampropeltis polyzona</i> *	1	1	5	7
<i>Lampropeltis splendida</i>	4	5	3	12
<i>Leptophis diplotropis</i> *	5	5	4	14
<i>Masticophis bilineatus</i>	2	5	4	11
<i>Masticophis flagellum</i>	1	3	4	8
<i>Masticophis mentovarius</i>	1	1	4	6
<i>Masticophis taeniatus</i>	1	5	4	10
<i>Opheodrys vernalis</i>	3	8	3	14
<i>Pantherophis bairdi</i>	4	7	4	15
<i>Pantherophis emoryi</i>	3	6	4	13
<i>Pituophis catenifer</i>	4	1	4	9
<i>Pituophis deppei</i> *	5	5	4	14
<i>Rhinocheilus lecontei</i>	1	3	4	8
<i>Salvadora bairdi</i> *	5	6	4	15
<i>Salvadora grahamiae</i>	4	2	4	10
<i>Senticolis triaspis</i>	2	1	3	6
<i>Sonora semiannulata</i>	1	1	3	5
<i>Tantilla atriceps</i>	2	7	2	11
<i>Tantilla cucullata</i>	4	6	2	12
<i>Tantilla hobartsmithi</i>	3	6	2	11
<i>Tantilla nigriceps</i>	3	6	2	11
<i>Tantilla wilcoxi</i>	2	6	2	10
<i>Tantilla yaquia</i>	2	6	2	10
<i>Trimorphodon tau</i> *	5	4	4	13
<i>Trimorphodon wilkinsonii</i>	4	7	4	15
<i>Diadophis punctatus</i>	1	1	2	4
<i>Geophis dugesii</i> *	5	6	2	13
<i>Heterodon kennerlyi</i>	3	4	4	11
<i>Hypsiglena chlorophaea</i>	1	5	2	8
<i>Hypsiglena jani</i>	1	3	2	6
<i>Imantodes gemmistratus</i>	1	3	2	6
<i>Leptodeira splendida</i> *	5	5	4	14
<i>Rhadinaea hesperia</i> *	5	3	2	10
<i>Rhadinaea laureata</i> *	5	5	2	12
<i>Micruroides euryxanthus</i>	4	6	5	15
<i>Micrurus distans</i> *	5	4	5	14
<i>Rena dulcis</i>	4	8	1	13
<i>Rena humilis</i>	4	3	1	8
<i>Nerodia erythrogaster</i>	3	4	4	11
<i>Storeria storerioides</i> *	5	4	2	11
<i>Thamnophis cyrtopsis</i>	2	1	4	7
<i>Thamnophis elegans</i>	3	7	4	14
<i>Thamnophis eques</i>	2	2	4	8
<i>Thamnophis errans</i> *	5	7	4	16
<i>Thamnophis marcianus</i>	1	5	4	10
<i>Thamnophis sirtalis</i>	3	7	4	14
<i>Agkistrodon laticinctus</i>	3	6	5	14
<i>Crotalus atrox</i>	1	3	5	9
<i>Crotalus basiliscus</i> *	5	6	5	16

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Table 17 (continued). Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Chihuahua, Mexico that are assigned to the IUCN Least Concern (LC) category. Non-native taxa are excluded. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic distribution	Ecological distribution	Reproductive mode/ Degree of persecution	Total score
<i>Crotalus molossus</i>	2	1	5	8
<i>Crotalus pricei</i>	2	7	5	14
<i>Crotalus scutulatus</i>	2	4	5	11
<i>Crotalus viridis</i>	1	6	5	12
<i>Crotalus willardi</i>	2	6	5	13
<i>Chrysemys picta</i>	3	8	3	14
<i>Kinosternon flavescens</i>	3	6	3	12
<i>Kinosternon hirtipes</i>	2	5	3	10
<i>Kinosternon integrum</i> *	5	3	3	11
<i>Apalone spinifera</i>	3	6	6	15

and can be relegated to the VU category, the 12 species with an EVS of 15 or 16 can go in the EN category, and the two species with an EVS of 17 or 19 should be in the CR category.

Relative Herpetofaunal Priority

Johnson et al. (2015a) introduced the concept of Relative Herpetofaunal Priority (RHP) in an MCS study on the herpetofauna of the Mexican state of Chiapas. This device involves a simple means of determining the relative conservation significance of the herpetofauna of any geographical entity (e.g., a physiographic region, municipality, or state), and comprises two moieties: (1) ascertaining the proportion of country endemic species (and, in certain cases, state endemic species); and (2) determining the absolute number of high EVS category species in each regional herpetofauna. We provide the data for these two approaches in Tables 18 and 19.

The relative numbers of country and state endemics range from three in the Sierras Plegadas del Norte to 57 in the Gran Meseta y Cañones Chihuahuenses (Table 18). The average number of endemic species in the nine physiographic regions is 16.4. Thus, three of the nine regions have values above this mean, including the Sierras y Cañadas del Norte (with 23 endemics), Gran Meseta y Cañones Duranguenses (26), and the Gran Meseta y Cañones Chihuahuenses (57). Interestingly, these three regions are the only ones in the state that house the single state endemic (*Plestiodon multilineatus***).

Using the other RHP measure (i.e., relative number of high vulnerability species), from a conservation perspective the Gran Meseta y Cañones Chihuahuenses is the most significant region (Table 19). The number of high EVS species in each region ranges from 11 to 46, with a mean of 21.1. Four of the nine regions have values above this mean, including the Bolsón de Mapimí (with 22 high EVS species), Sierras y Cañadas del Norte (25), Gran Meseta y Cañones Chihuahuenses (46), and the Gran Meseta y Cañones Duranguenses (22).

The comparable ranks indicated based on the two RHP measures (endemic species rank on left and high EVS species rank on the right) are as follows (see Tables 18 and 19):

- Gran Meseta y Cañones Chihuahuenses (1—1)
- Gran Meseta y Cañones Duranguenses (2—3)
- Sierras y Cañadas de Norte (3—2)

- Sierras y Llanuras de Durango (4—8)
- Sierra y Llanuras Tarahumaras (5—5)
- Bolsón de Mapimí (6—3)
- Llanuras y Médanos del Norte (7—4)
- Llanuras y Sierras Volcánicas (8—6)
- Sierras Plegadas del Norte (9—7)

There is little agreement in the respective ranges for the two RHP measures, except that Gran Meseta y Cañones Chihuahuenses occupies the 1st rank with both measures and Sierra y Llanuras Tarahumaras the 5th rank.

The 58 endemic species in the Gran Meseta y Cañones Chihuahuenses include 12 anurans, three salamanders, 17 lizards, 23 snakes, and three turtles. This region also harbors the following 49 high-vulnerability species:

- Ambystoma rosaceum** (14)
- Ambystoma silvense** (14)
- Aspidoscelis exsanguis* (14)
- Aspidoscelis inornata* (14)
- Aspidoscelis marmorata* (14)
- Aspidoscelis tessellata* (14)
- Aspidoscelis uniparens* (15)
- Barisia ciliaris** (15)
- Barisia imbricata** (14)
- Barisia levicollis** (15)
- Boa sigma** (15)
- Craugastor tarahumaraensis** (17)
- Crotalus basiliscus** (16)
- Crotalus pricei* (14)
- Ctenosaura macrolopha** (19)
- Eleutherodactylus interorbitalis** (15)
- Gopherus evgoodei** (18)
- Holbrookia approximans** (14)
- Incilius mccoysi** (14)
- Isthmura sierraoccidentalis** (17)
- Kinosternon sonoriense* (14)
- Lampropeltis alterna* (14)
- Leptodeira splendida** (14)
- Leptophis diplotropis** (14)
- Lithobates lemosespinali** (14)
- Mastigodryas cliftoni** (14)
- Micruroides euryxanthus* (15)
- Micrurus distans** (14)
- Opheodrys vernalis* (14)
- Phyllodactylus saxatilis** (16)

*Pituophis deppei** (14)
*Plestiodon multilineatus*** (16)
Plestiodon multivirgatus (14)
*Plestiodon parviauriculatus** (15)
*Rena dugesii** (14)
*Salvadora bairdi** (15)
Salvadora deserticola (14)
*Sceloporus albiventris** (16)
*Sceloporus lemosespinali** (16)
Sceloporus virgatus (15)
*Sonora aemula** (16)
*Sympholis lippiens** (14)
*Terrapene nelsoni** (18)
Thamnophis elegans (14)
*Thamnophis errans** (16)
*Thamnophis melanogaster** (15)
Thamnophis sirtalis (14)
*Thamnophis unilabialis** (16)
*Tropidodipsas repleta** (17)

These 49 species include four anurans, three salamanders, 17 lizards, 22 snakes, and three turtles. Thirty-four (69.4%) of these species are endemic to Mexico (33 species) or to the state of Chihuahua (one species). Their EVS values range from 14 to 19.

The 26 endemic species in the Gran Meseta y Cañones Duranguenses include four anurans, one salamander, 10 lizards, 10 snakes, and one turtle. This region also supports the following 22 high EVS species:

*Ambystoma rosaceum** (14)
Aspidoscelis exsanguis (14)
Aspidoscelis inornata (14)
*Boa sigma** (15)
Crotalus pricei (14)

*Ctenosaura macrolopha** (19)
*Incilius mccoysi** (14)
*Leptodeira splendida** (14)
*Mastigodryas cliftoni** (14)
Opheodrys vernalis (14)
*Plestiodon multilineatus*** (16)
Plestiodon multivirgatus (14)
*Plestiodon parviauriculatus** (15)
Salvadora deserticola (14)
*Sceloporus albiventris** (16)
*Sceloporus lemosespinali** (16)
*Sonora aemula** (16)
*Terrapene nelsoni** (18)
Thamnophis elegans (14)
*Thamnophis errans** (16)
Thamnophis sirtalis (14)
*Thamnophis unilabialis** (16)

These 22 species include one anuran, one salamander, eight lizards, 11 snakes, and one turtle. Thirteen (59.1%) of these species are endemic to Mexico and one (4.5%) is endemic to Chihuahua. Their EVS values range from 14 to 19.

The 23 endemic species in the Sierras y Cañadas del Norte include six anurans, three salamanders, eight lizards, and six snakes. This region also contains the following 26 high EVS species:

*Ambystoma rosaceum** (14)
*Ambystoma silvense** (14)
Aspidoscelis exsanguis (14)
Aspidoscelis uniparens (15)
*Barisia imbricata** (14)
*Barisia levicollis** (15)
*Craugastor tarahumaraensis** (17)

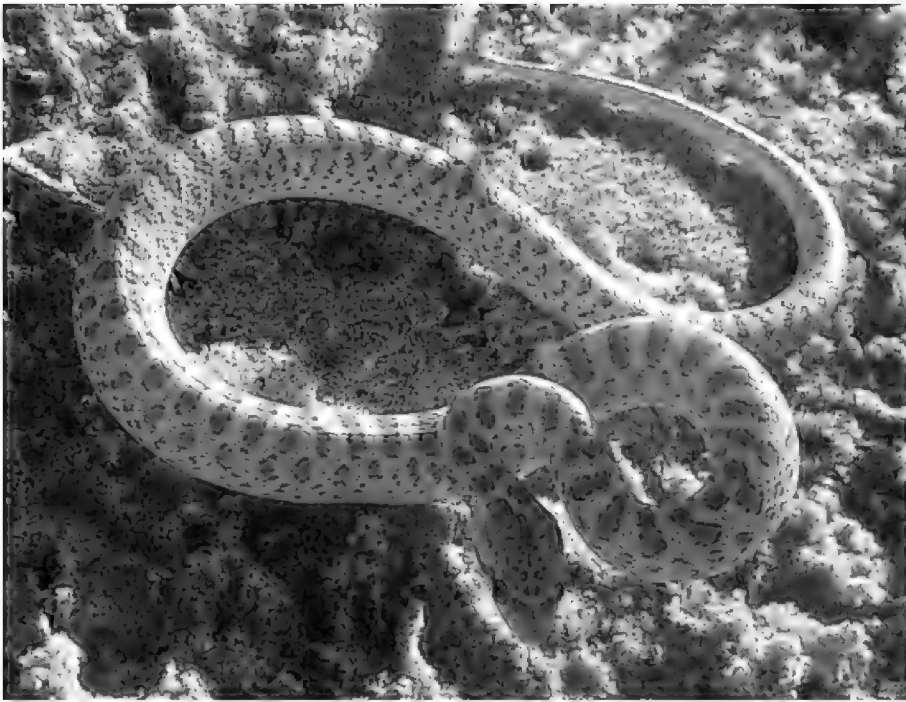
Table 18. Number of herpetofaunal species in the four distributional status categories among the nine physiographic regions of Chihuahua, Mexico. Rank is based on the number of country and state endemics. The numbers in parentheses in the rank order column represent the sum of the country endemic and state endemic numbers.

Physiographic region	Distributional category				Total	Rank order
	Non-endemics	Country endemics	State endemics	Non-natives		
Llanuras y Médanos del Norte LMN	75	5	—	1	81	7 (5)
Sierras Plegadas del Norte SPN	60	3	—	1	64	9 (3)
Bolsón de Mapimí BDM	71	7	—	2	80	6 (7)
Llanuras y Sierras Volcánicas LSV	69	4	—	2	75	8 (4)
Sierras y Cañadas del Norte SCN	56	22	1	—	79	3 (23)
Sierras y Llanuras Tarahumaras SLT	50	11	—	—	61	5 (11)
Gran Meseta y Cañones Chihuahuenses GMCC	71	56	1	—	128	1 (57)
Sierras y Llanuras de Durango SLD	46	12	—	—	58	4 (12)
Gran Meseta y Cañones Duranguenses GMCD	33	25	1	—	59	2 (26)

Table 19. Number of herpetofaunal species in the three EVS categories in nine physiographic regions in Chihuahua, Mexico. Rank is determined by the relative number of high EVS species. Non-native species are excluded.

Physiographic region	Low	Medium	High	Total	Rank order
Llanuras y Médanos del Norte LMN	24	37	18	79	4
Sierras Plegadas del Norte SPN	21	28	13	62	7
Bolsón de Mapimí BDM	24	31	22	77	3
Llanuras y Sierras Volcánicas LSV	25	31	16	72	6
Sierras y Cañada del Norte SCN	19	34	25	78	2
Sierras y Llanuras Tarahumaras SLT	16	27	17	60	5
Gran Meseta y Cañones Chihuahuenses GMCC	28	53	46	127	1
Sierras y Llanuras de Durango SLD	18	28	11	57	8
Gran Meseta y Cañones Duranguenses GMCD	13	24	22	59	3

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No. 41. *Arizona elegans* Kennicott, 1859. The distribution of the Glossy Snake “extends from central California, southern Nevada, southern Utah, southwestern and eastern Colorado, and southeastern Nebraska southward through southern California, Arizona, New Mexico, Kansas, Oklahoma, and Texas, in the United States, and in Mexico in northern Baja California, Sinaloa, Aguascalientes, and in the Chihuahuan Desert, including western San Luis Potosí” (Lemos-Espinal and Dixon, 2013: 170–171). This juvenile was photographed in sandy soil in a somewhat stable sand dune at Médanos de Samalayuca, in Juárez, Chihuahua. Wilson et al. (2013a) ascertained its EVS as 5, placing it in the lower portion of the low vulnerability category. The IUCN designated its conservation status as Least Concern, but this species is not listed by SEMARNAT. *Photo by Rubén F. Alvidrez Heredia.*



No. 42. *Lampropeltis knoblochi* Taylor, 1940. The Chihuahuan Mountain Kingsnake “appears to be limited to the Sierra Madre Occidental of Sonora, Chihuahua, Sinaloa, and Durango, and Madrean Sky Islands in southeastern Arizona and southwestern New Mexico...” (Lemos-Espinal et al. 2013: 103). This individual was photographed on a rock with moss and lichens near Cascada de Basaseachi National Park, where there is pine-oak and gallery forest vegetation, in the municipality of Ocampo, Chihuahua. Wilson et al. (2013a) calculated its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. *Photo by Ramón Isaac Miramontes Cinco.*



No. 43. *Leptophis diplotropis* (Günther, 1872). The Pacific Coast Parrot Snake is found at “low altitudes on Pacific slopes from southern Sonora and southwestern Chihuahua (canyons of the Sierra Tarahumara) to the Isthmus of Tehuantepec, extending some distance up the Río Santiago valley” (Lemos-Espinal et al. 2013: 105). This individual was photographed in low deciduous forest at Urique, Chihuahua. Wilson et al. (2013a) determined its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN assessed its conservation status as Least Concern, but this species is listed as Threatened by SEMARNAT. *Photo by Sebastián Ochoa Rodríguez.*



No. 44. *Masticophis flagellum* (Shaw, 1802). The distribution of the Coachwhip “extends across the southern half of the United States, and in Mexico southward through Baja California, west of the Sierra Madre Occidental to southern Sinaloa, and east of the Sierra Madre Occidental to northern Jalisco and Querétaro” (Lemos-Espinal and Dixon 2013: 199–200). This individual was photographed in a crop field on the old road to the city of Meoqui, Chihuahua. Wilson et al. (2013a) calculated its EVS as 8, placing it in the upper portion of the low vulnerability category. The IUCN determined its conservation status as Least Concern, but this species is considered as Threatened by SEMARNAT. *Photo by Leonardo Hernández Escudero.*

Crotalus pricei (14)
Heloderma suspectum (15)
*Holbrookia approximans** (14)
*Incilius mccoysi** (14)
*Isthmura sierraoccidentalis** (17)
Kinosternon sonoriense (14)
Opheodrys vernalis (14)
*Plestiodon multilineatus*** (16)
Plestiodon multivirgatus (14)
*Plestiodon parviauriculatus** (15)
*Salvadora bairdi** (15)
Salvadora deserticola (14)
*Sceloporus lemosespinali** (16)
Sceloporus virgatus (15)
Thamnophis elegans (14)
*Thamnophis errans** (16)
*Thamnophis melanogaster** (15)
Thamnophis sirtalis (14)
*Thamnophis unilabialis** (16)

These 26 species include two anurans, three salamanders, 11 lizards, nine snakes, and one turtle. Fourteen (53.8%) of these species are country endemics and one (3.8%) is a state endemic. Their EVS values range from 14 to 17.

Twelve endemic species inhabit the Sierras y Llanuras de Durango, including three anurans, two salamanders, five lizards, and two snakes. This region also harbors the following 12 high EVS species:

*Ambystoma silvense** (14)
Aspidoscelis exsanguis (14)
Aspidoscelis tessellata (14)
Aspidoscelis uniparens (15)
*Barisia ciliaris** (15)
Cophosaurus texanus (14)
*Craugastor tarahumaraensis** (17)
*Holbrookia approximans** (14)
Salvadora deserticola (14)
*Sceloporus albiventris** (16)
*Thamnophis errans** (16)
*Thamnophis unilabialis** (16)

These 12 species include one anuran, one salamander, seven lizards, and three snakes. Seven (58.3%) of these species are country endemics. Their EVS values range from 14 to 17.

Eleven endemic species occur in the Sierras y Llanuras Tarahumaras, including three anurans, two salamanders, five lizards, and one snake. This region also contains the following 18 high EVS species:

*Ambystoma rosaceum** (14)
Aspidoscelis marmorata (14)
*Ambystoma silvense** (14)
Aspidoscelis tessellata (14)
*Barisia imbricata** (14)
Aspidoscelis uniparens (15)
*Barisia levicollis** (15)
Salvadora deserticola (14)
*Holbrookia approximans** (14)
Thamnophis elegans (14)
*Sceloporus lemosespinali** (16)
*Thamnophis errans** (16)

Sceloporus virgatus (15)
Thamnophis sirtalis (14)
Aspidoscelis exsanguis (14)
Crotalus pricei (14)
Aspidoscelis inornata (14)
Kinosternon sonoriense (14)

These 18 species include two salamanders, 10 lizards, five snakes, and one turtle. Seven (38.9%) of these are country endemics. Their EVS values range from 14 to 16.

The Bolsón de Mapimí region houses seven endemic species, including one anuran, one salamander, two lizards, one snake, and two turtles. This region also contains the following 21 high EVS species:

*Ambystoma rosaceum** (14)
Apalone spinifera (15)
Aspidoscelis exsanguis (14)
Aspidoscelis inornata (14)
Aspidoscelis marmorata (14)
Aspidoscelis tessellata (14)
Aspidoscelis uniparens (15)
Bogertophis subocularis (14)
Chrysemys picta (14)
Cophosaurus texanus (14)
*Gopherus flavomarginatus** (19)
*Holbrookia approximans** (14)
*Kinosternon durangoense** (16)
*Pituophis deppei** (14)
Salvadora deserticola (14)
Terrapene ornata (15)
Thamnophis elegans (14)
Thamnophis sirtalis (14)
Trachemys gaigeae (18)
Trimorphodon wilkinsonii (15)
*Uma paraphygae** (17)

These 21 species include one salamander, eight lizards, six snakes, and six turtles. Six (28.6%) of these species are country endemics. Their EVS values range from 14 to 19.

The Llanuras y Médanos del Norte harbors five country endemics, including two anurans, one lizard, and two turtles. This region also contains the following 18 high EVS species:

Apalone spinifera (15)
Aspidoscelis exsanguis (14)
Aspidoscelis inornata (14)
Aspidoscelis marmorata (14)
Aspidoscelis tessellata (14)
Aspidoscelis uniparens (15)
Chrysemys picta (14)
Cophosaurus texanus (14)
*Holbrookia approximans** (14)
Kinosternon sonoriense (14)
Plestiodon multivirgatus (14)
Salvadora deserticola (14)
Sceloporus virgatus (15)
*Terrapene nelsoni** (18)
Terrapene ornata (15)

Thamnophis sirtalis (14)
Trachemys gaigeae (18)
Trimorphodon wilkinsonii (15)

These 18 species comprise nine lizards, three snakes, and six turtles. Only two are country endemics. Their EVS values range from 14 to 18.

The Llanuras y Sierras Volcánicas harbors four country endemics, including one anuran, one lizard, and two turtles. This region also contains the following 17 high EVS species:

Agkistrodon laticinctus (15)
Apalone spinifera (15)
Aspidoscelis exsanguis (14)
Aspidoscelis inornata (14)
Aspidoscelis marmorata (14)
Aspidoscelis tessellata (14)
Aspidoscelis uniparens (15)
Bogertophis subocularis (14)
Coleonyx brevis (14)
Cophosaurus texanus (14)
*Gopherus flavomarginatus** (19)
*Holbrookia approximans** (14)
Kinosternon durangoense (16)
Lampropeltis alterna (14)
Pantherophis bairdi (15)
Salvadora deserticola (14)
Terrapene ornata (15)

These 17 species consist of eight lizards, five snakes, and four turtles. Only two of these species are country endemics. Their EVS values range from 14 to 19.

The Sierras Plegadas del Norte harbors three country endemics, including one anuran and two lizards. This region also supports the following 14 high EVS species:

Apalone spinifera (15)
Aspidoscelis exsanguis (14)
Aspidoscelis inornata (14)
Aspidoscelis marmorata (14)
Aspidoscelis tessellata (14)
Aspidoscelis uniparens (15)
Bogertophis subocularis (14)
Coleonyx brevis (14)
Cophosaurus texanus (14)
*Holbrookia approximans** (14)
Salvadora deserticola (14)
Terrapene ornata (15)
Trachemys gaigeae (18)
Trimorphodon wilkinsonii (15)

These 14 species comprise eight lizards, three snakes, and three turtles. Only one of these species is a country endemic. Their EVS values range from 14 to 18.

Of the 183 native herpetofaunal species of herpetofauna in Chihuahua, 62 are endemics (33.9%) and 64 (35.0%) are high EVS species. Their numbers in the nine physiographic regions we recognize range from 12 to 49. These allocations are important for developing management plans in the protected areas of Chihuahua, as we discuss in the following section

Natural Protected Areas in Chihuahua

Biodiversity decline is one of the most significant global environmental problems affecting our planet. This problem is so extensive that biologists have described it as the sixth great extinction (Kolbert 2014). As with all global environmental problems, biodiversity decline is characterized by the following features: (1) insofar as we know, this is a worldwide problem that affects all components of the biosphere; (2) even though the decline is thought to be extensive, the actual extent is currently unknown and might never be known; (3) the problem is considered to be the result of the impact of an uncontrolled human population acting on limited supplies of resources, thereby producing widespread pollution; (4) the problem is expected to continue exacerbating until a currently incompletely understood tipping point is reached, forecasting an expected biospheric collapse; and (5) the basis for the problem is in the widespread affliction of humans by “a social disease termed anthropocentrism, for which the symptoms arise from denying the reality of natural law” (Leyte-Manrique et al. 2022: 167; also see Wilson and Lazcano 2019).

Conservation biologists are faced with attempting to control the short- and long-term manifestations of biodiversity decline. These biologists, however, are trying to fight this global environmental problem with primitive weapons that are in short supply.

The most basic problem conservation biologists face is a lack of knowledge of the true global extent of biodiversity, not even to the nearest order of magnitude (Wilson 2002). Thus, conservation biologists depend upon systematic biologists, who are attempting, with limited resources, to answer a fundamental question...Just how many species of organisms presently occupy our planet? Without an answer to this seemingly simple question, it is impossible to produce a suitable answer to more complex questions such as...How are all the world's creatures organized into ecosystems? Which of these ecosystems are most endangered by the actions of human beings? How close is humanity to bringing the biosphere (known and unknown) to the tipping point of biospheric collapse? Which organisms (known or unknown) lie on the cusp of that tipping point? As noted by Wilson (2002: 21), “the biospheric membrane that covers Earth, and you and me...is a miracle we have been given...” It is also “our tragedy, because a large part of it is being lost forever before we learn what it is and the best means by which it can be savored and used.”

So, from the statements in the earlier part of this section, it should be evident that conservation biologists are able to make only minimal inroads in finding answers to these elemental questions about the natural world. One of the tools these biologists can use is to establish natural protected areas in an effort to provide some level of protection, hopefully for perpetuity, to particular groups of organisms occupying particular portions of ecosystems. Amphibians and reptiles are rarely the target organisms of such efforts, so in this paper it is important to assess the level of protection that the herpetofauna of Chihuahua presently has. For this reason, we present a summary of the known information on the natural protected areas of this state in Table 20.

Eleven natural protected areas (NPAs) occur in Chihuahua (Table 20). Two of these areas are biosphere reserves (Janos and Mapimi), two are national parks (Cascada de Bassascachic and Cumbres de Majalca), six are Flora and Fauna Protected Areas (Papigochic, Tutuaca, Cerro Mohinora, Médanos de Samalayuca, Campo Verde, and Cañon de Santa Elena), and one is a National Monument (Río Bravo del Norte). All 11 of these NPAs are under federal or national/federal jurisdiction.

These 11 NPAs protect portions of the following physiographic regions in Chihuahua: Bolsón de Mapimí (BDM), Gran Meseta y Cañones Chihuahuenses (GMCC), Gran Meseta y Cañones Duranguenses (GMCD), Llanuras y Médanos del Norte (LMN), Llanuras y Sierras Volcánicas (LSV), Sierras y Cañadas del Norte (SCN), Sierras y Llanuras Tarahumaras (SLT), and Sierras Plegadas del Norte (SPN). The only physiographic region with no representation in the protected area system in Chihuahua is the Sierras y Llanuras de Durango (SLD).

These NPAs were established from 1937 to 2015, with seven decreed during the present century (2001–2015). They range in area from 2,175 to 526,482.43 ha, with an average area of 181,076.06 ha (= 1,810.76 km² or 699.14 mi²).

Only two of the 11 NPAs are unoccupied by landowners. Seven of these NPAs involve ejidos (which are communally held and collectively farmed land) and private ownership, one includes ejidos and rural communities, and one encompasses ejidos, private ownership, and national territory.

Of considerable significance is that all 11 of the NPAs have available management programs. In addition, and even more significantly, is that 10 of the 11 have completed herpetofaunal surveys.

Based on these surveys and other information, we were able to determine the herpetofaunal species found within the 11 NPAs (summarized in Table 21). Of the 40 species of amphibians in Chihuahua, 26 (65.0%) have been recorded in one or more of the NPAs, including 24 of 35 anuran species (68.6%) and two of five salamander species (40.0%). Of the 146 species of reptiles in the state, 99 (67.8%) have been recorded in one or more of the NPAs, including 90 of 133 squamates (67.7%) and nine of 13 turtles (69.2%). For the total herpetofauna of 186 species, 125 species (67.2%) are known from the compendium of 11 NPAs. Consequently, 61 species (32.8%) still have not been recorded from any of these NPAs. Of the 125 species of amphibians and reptiles presently recorded from the NPAs in the state of Chihuahua, 94 (75.2%) are non-endemic species, 28 (22.4%) are country endemics, one (0.8%) is a state endemic, and two (1.6%) are non-natives (Table 22).

The number of species recorded for each of the 11 NPAs ranges from 20 to 71 (Table 21). The lowest number is for Fauna and Flora Protected Area (FFPA) Campo Verde, which is the only NPA in Chihuahua for which no herpetofaunal survey is available. The highest number is from National Park Cascada de Bassascachic, which interestingly is one of the smallest NPAs in the state at 5,802.85 ha, but it also is one of the two NPAs that is not occupied by landowners (Table 20). The largest of the NPAs in Chihuahua, Biosphere Reserve Janos, is known to support 50 species of amphibians and reptiles.

Table 20. Characteristics of the Natural Protected Areas (NPAs) in Chihuahua, Mexico. Abbreviations for the category types of NPAs are: FFPA = Flora and Fauna Protected Area; NP = National Park; BR = Biosphere Reserve; and NM = Natural Monument. Abbreviations for services available in facilities are: A = administrative services; R = park guards; S = system of pathways; and V = facilities for visitors. Data were obtained from: Biosphere Reserves in Latin America and the Caribbean (<https://en.unesco.org/biosphere/lac>; Accessed: 19 March 2024).

Name	Category	Date of decree	Area (ha)	Municipalities of Chihuahua	Jurisdiction	Physiographic regions	Facilities available (A, R, S, V)	Occupied by landowners	Herpetofaunal survey completed	Management program available
Papigochic	FFPA	29 January 2003	222,763.85	Guerrero, Bocoyna, Ocampo and Temósachic	Federal	SCN, GMCC and SLT	A, R	Ejidos and private ownership	Yes	Yes
Tutuaca	FFPA	27 December 2001	436,985.67	Temósachic, Guerrero, Matachi, Madera, Morris and Ocampo	Federal	SCN, GMCC and SLT	A, R	Ejidos and rural communities	Yes	Yes
Cerro Mohinora	FFPA	10 July 2015	9,126.36	Guadalupe y Calvo	Federal	GMCD	A, R	Ejidos and private ownership	Yes	Yes
Cascada de Bassascachic	National Park	2 February 1981	5,802.85	Ocampo	Federal	GMCC	A, R	Not occupied	Yes	Yes
Cumbres de Majalca	National Park	1 September 1939	4,701.28	Chihuahua and Riva Palacio	Federal	SLT	A, R	Ejidos, public and private ownership	Yes	Yes
Janos	Biosphere Reserve	7 June 1937	526,482.43	Janos	Federal	LMN and SCN	A, R, S, V	Ejidos and private ownership	Yes	Yes
Médanos de Samalayuca	FFPA	5 June 2009	56,134.38	Juárez and Guadalupe DB	Federal	LMN and SPN	A, R, S, V	Ejidos and private ownership	Yes	Yes
Campo Verde	FFPA	29 January 22003	108,067.47	Madera and Casas Grandes	Federal	SCN	A, R	Ejidos and private ownership	No	No
Cañon de Santa Elena	FFPA	7 November 1994	277,209.72	Manuel Benavides and Ojinaga	National/Federal	LSV	A, R, S, V	Ejidos, private ownership and national territory	Yes	Yes
Mapimi	Biosphere Reserve*	27 November 2000	342,387.99	Jiménez	Federal	BDM	A, R, S, V	Ejidos and small private ownership	Yes	Yes

* The Mapimí Biosphere Reserve was the first biosphere reserve in Mexico, designated in 1977 by the “Man and Biosphere” (MAB) program of UNESCO.

The herpetofauna of Chihuahua, Mexico

Table 21. Distribution of herpetofaunal species in the Natural Protected Areas of Chihuahua, Mexico. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species. The numbers for the Natural Protected Areas are: 1 = Papigochic, 2 = Tutuaca, 3 = Mohinora, 4 = Bassaseachic, 5 = Cumbres de Majalca, 6 = Janos, 7 = Médanos de Samalayuca, 8 = Campo Verde, 9 = Cañón de Santa Elena, 10 = Mapimí, and 11 = Rio Bravo.

Taxon	Natural Protected Area										
	1	2	3	4	5	6	7	8	9	10	11
Amphibia (26 species)											
Anura (24 species)											
Bufonidae (10 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Anaxyrus cognatus</i>	+	+		+	+	+	+		+	+	
<i>Anaxyrus debilis</i>				+		+	+		+	+	+
<i>Anaxyrus mexicanus</i> *		+						+			
<i>Anaxyrus punctatus</i>	+	+	+	+	+	+	+	+	+	+	+
<i>Anaxyrus speciosus</i>											
<i>Anaxyrus woodhousii</i>		+	+	+	+	+	+	+			
<i>Incilius alvarius</i>				+		+	+	+			
<i>Incilius mazatlanensis</i> *		+	+					+			
<i>Incilius mccoysi</i> *				+							
<i>Incilius occidentalis</i>	+							+			
Craugastoridae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Craugastor augusti</i>				+		+					
<i>Craugastor tarahumaraensis</i> *	+	+	+								
Hylidae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Dryophytes arenicolor</i>	+	+	+	+	+	+		+	+		
<i>Dryophytes wrightorum</i>	+	+	+	+	+			+			
Microhylidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Gastrophryne olivacea</i>		+	+	+	+				+	+	
Ranidae (6 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Lithobates berlandieri</i>	+		+	+			+		+		+
<i>Lithobates catesbeianus</i> ***						+	+	+	+		
<i>Lithobates chiricahuensis</i>			+			+		+	+		
<i>Lithobates lemosespinali</i> *								+			
<i>Lithobates tarahumarae</i>			+	+	+			+	+		
<i>Lithobates yavapaiensis</i>				+					+		
Scaphiopodidae (3 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Scaphiopus couchii</i>				+		+	+		+	+	+
<i>Spea bombifrons</i>				+		+	+				
<i>Spea multiplicata</i>						+	+			+	
Caudata (2 species)											
Ambystomatidae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Ambystoma rosaceum</i> *	+	+	+			+		+			
<i>Ambystoma velasci</i> *	+	+	+		+			+			
Reptilia (99 species)											
Squamata (90 species)											
Anguidae (4 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Barisia imbricata</i>	+							+			
<i>Barisia levicollis</i> *	+	+									
<i>Elgaria kingii</i>		+	+	+	+						
<i>Gerrhonotus infernalis</i>									+		
Crotaphytidae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Crotaphytus collaris</i>				+	+	+	+		+	+	+
<i>Gambelia wislizenii</i>				+			+		+	+	+
Eublepharidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Coleonyx brevis</i>				+			+		+	+	+
Gekkonidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Hemidactylus turcicus</i> ***											+
Iguanidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Ctenosaura macrolopha</i> *			+								
Phrynosomatidae (19 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Cophosaurus texanus</i>				+	+	+	+		+	+	+
<i>Holbrookia approximans</i> *							+				
<i>Holbrookia maculata</i>				+	+	+	+		+	+	
<i>Phrynosoma cornutum</i>	+	+		+	+	+	+		+	+	+
<i>Phrynosoma hernandesi</i>			+	+	+						
<i>Phrynosoma modestum</i>				+		+	+		+	+	+
<i>Phrynosoma orbiculare</i> *	+		+	+				+			
<i>Sceloporus clarkii</i>			+								
<i>Sceloporus cowlesi</i>							+				
<i>Sceloporus jarrovii</i>	+	+	+	+	+						

Table 21 (continued). Distribution of herpetofaunal species in the Natural Protected Areas of Chihuahua, Mexico. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species. The numbers for the Natural Protected Areas are: 1 = Papigochic, 2 = Tutuaca, 3 = Mohinora, 4 = Bassaseachic, 5 = Cumbres de Majalca, 6 = Janos, 7 = Médanos de Samalayuca, 8 = Campo Verde, 9 = Cañón de Santa Elena, 10 = Mapimí, and 11 = Rio Bravo.

Taxon	Natural Protected Area										
	1	2	3	4	5	6	7	8	9	10	11
<i>Sceloporus magister</i>				+		+	+		+	+	+
<i>Sceloporus merriami</i>									+		+
<i>Sceloporus poinsettii</i>			+	+	+	+	+		+	+	
<i>Sceloporus slevini</i>				+							
<i>Sceloporus virgatus</i>				+							
<i>Uma paraphygas</i> *									+	+	
<i>Urosaurus ornatus</i>				+	+	+	+		+		
<i>Uta stansburiana</i>				+		+	+		+	+	
Scincidae (5 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Plestiodon bilineatus</i> *			+								
<i>Plestiodon multilineatus</i> **	+	+						+			
<i>Plestiodon obsoletus</i>				+		+	+		+	+	
<i>Plestiodon parviauriculatus</i> *		+	+					+			
<i>Plestiodon tetragrammus</i>				+							
Teiidae (7 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Aspidoscelis costatus</i> *			+								
<i>Aspidoscelis exsanguis</i>				+	+	+	+				
<i>Aspidoscelis gularis</i>									+		+
<i>Aspidoscelis inornata</i>				+		+	+		+	+	
<i>Aspidoscelis marmorata</i>							+		+	+	+
<i>Aspidoscelis tessellata</i>							+		+		
<i>Aspidoscelis uniparens</i>				+	+	+	+				
Colubridae (25 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Arizona elegans</i>				+		+	+		+	+	
<i>Bogertophis subocularis</i>									+	+	
<i>Drymarchon melanurus</i>			+								
<i>Gyalopion canum</i>				+		+	+				
<i>Lampropeltis knoblochi</i>				+	+						
<i>Lampropeltis splendida</i>							+				
<i>Masticophis bilineatus</i>				+							
<i>Masticophis flagellum</i>			+	+	+		+		+	+	+
<i>Masticophis taeniatus</i>					+	+	+		+	+	
<i>Opheodrys vernalis</i>				+							
<i>Pantherophis bairdi</i>									+		
<i>Pantherophis emoryi</i>							+				
<i>Pituophis catenifer</i>				+	+	+	+		+	+	
<i>Pituophis deppei</i> *	+								+		
<i>Rhinocheilus lecontei</i>				+		+	+		+	+	
<i>Salvadora bairdi</i> *									+		
<i>Salvadora deserticola</i>				+		+	+		+		+
<i>Salvadora grahamiae</i>				+		+					
<i>Senticolis triaspis</i>			+								
<i>Sonora semiannulata</i>				+		+	+		+	+	
<i>Tantilla atriceps</i>				+			+				
<i>Tantilla hobartsmithi</i>							+				
<i>Tantilla nigriceps</i>				+		+					
<i>Tantilla wilcoxi</i>			+	+	+						
<i>Trimorphodon tau</i> *				+							
Dipsadidae (6 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Diadophis punctatus</i>			+	+		+	+		+	+	
<i>Geophis dugesii</i> *			+								
<i>Heteredon kennerlyi</i>				+		+	+		+	+	
<i>Hypsiglena chlorophaea</i>				+							
<i>Hypsiglena jani</i>						+	+		+		
<i>Leptodeira splendida</i> *			+								
Leptotyphlopidae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Rena dulcis</i>				+		+	+				
<i>Rena humilis</i>				+		+	+		+	+	
Natricidae (9 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Storeria storerioides</i> *			+	+							
<i>Thamnophis cyrtopsis</i>	+	+	+	+	+	+	+		+		+
<i>Thamnophis elegans</i>			+								
<i>Thamnophis eques</i>	+		+	+							
<i>Thamnophis errans</i> *	+			+				+			

The herpetofauna of Chihuahua, Mexico

Table 21. Distribution of herpetofaunal species in the Natural Protected Areas of Chihuahua, Mexico. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Chihuahua; and *** = non-native species. The numbers for the Natural Protected Areas are: 1 = Papigochic, 2 = Tutuaca, 3 = Mohinora, 4 = Bassaseachic, 5 = Cumbres de Majalca, 6 = Janos, 7 = Médanos de Samalayuca, 8 = Campo Verde, 9 = Cañón de Santa Elena, 10 = Mapimí, and 11 = Rio Bravo.

Taxon	Natural Protected Area										
	1	2	3	4	5	6	7	8	9	10	11
<i>Thamnophis melanogaster</i> *								+			
<i>Thamnophis sirtalis</i>						+	+				
<i>Thamnophis unilabialis</i> *	+		+	+							
Viperidae (8 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Crotalus atrox</i>						+	+		+	+	+
<i>Crotalus lepidus</i>	+	+	+	+	+	+	+		+	+	
<i>Crotalus molossus</i>	+	+	+	+	+	+					
<i>Crotalus ornatus</i>							+		+	+	
<i>Crotalus pricei</i>	+	+	+	+							
<i>Crotalus scutulatus</i>				+	+	+	+		+	+	
<i>Crotalus viridis</i>						+	+		+		
<i>Crotalus willardi</i>	+	+	+	+							
Testudines (9 species)											
Emydidae (2 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Terrapene ornata</i>					+	+	+				
<i>Trachemys gaigeae</i>									+		
Kinosternidae (5 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Kinosternon durangoense</i> *										+	
<i>Kinosternon flavescens</i>						+			+		+
<i>Kinosternon hirtipes</i>	+	+		+	+				+		
<i>Kinosternon integrum</i> *		+									
<i>Kinosternon sonoriense</i>				+							
Testudinidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Gopherus flavomarginatus</i> *										+	
Trionychidae (1 species)	1	2	3	4	5	6	7	8	9	10	11
<i>Apalone spinifera</i>											+
Total (125 species)	25	24	38	71	31	50	55	20	55	37	22

In general, about two-thirds of the state’s herpetofauna has been recorded within the state’s NPAs, leaving about one-third unrecorded and unprotected. The one-third, or 61 species, still not recorded from any NPA include the following 11 anurans:

- Agalychnis dacnicolor**
- Eleutherodactylus interorbitalis**
- Eleutherodactylus marnockii*
- Gastrophryne mazatlanensis*
- Hypopachus variolosus*
- Lithobates cora*
- Lithobates magnaocularis**
- Lithobates pustulosus**
- Rhinella horribilis*
- Smilisca baudinii*
- Tlalocohyla smithi**

These unprotected species also include the following three salamanders:

- Ambystoma mavortium*
- Isthmura sierraoccidentalis**
- Ambystoma silvense**

The following 43 squamates are also included among the unprotected species:

- Agkistrodon bilineatus*
- Agkistrodon laticinctus*
- Aspidoscelis neomexicanus*
- Aspidoscelis sonora*

- Barisia ciliaris**
- Boa sigma**
- Conopsis nasus**
- Crotalus basiliscus**
- Drymobius margaritiferus*
- Gyalopion quadrangulare*
- Heloderma horridum**
- Heloderma suspectum*
- Holbrookia elegans*
- Imantodes gemmistratus*
- Indotyphlops braminus****
- Lampropeltis alterna*
- Lampropeltis polyzona*
- Leptophis diplotropis**
- Masticophis mentovarius*
- Mastigodryas cliftoni**
- Micruroides euryxanthus*
- Micrurus distans**
- Nerodia erythrogaster*
- Norops nebulosus**
- Oxybelis microphthalmus*
- Phyllodactylus saxatilis**
- Plestiodon callicephalus*
- Plestiodon multivirgatus*
- Rena dugesii**
- Rena segrega*
- Rhadinaea hesperia**
- Rhadinaea laureata**
- Salvadora lineata*
- Sceloporus albiventris*
- Sceloporus nelsoni**
- Sonora aemula**



No. 45. *Masticophis taeniatus* (Hallowell, 1852). The Striped Whipsnake “is distributed from Idaho and Washington in the northwestern United States through the Great Basin and Chihuahuan Desert south to the Mexican Plateau. In Mexico, it is known from Chihuahua, Coahuila, Durango, Zacatecas, Aguascalientes and northeastern Jalisco” (Heimes 2016: 111). This individual was photographed in pine forest at Teseachi ranch, in Bachíniva, Chihuahua. Wilson et al. (2013a) determined its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN indicated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Sara G. Sáenz González.*



No. 46. *Pituophis catenifer* (Blainville, 1835). The distribution of the Gopher Snake “extends from southwestern Canada to the Great Lakes region of the United States, and southward to include most of northern Mexico” (Lemos-Espinal and Dixon 2013: 207–208). This adult was photographed on the road to Rancho El Berrendo, in Ascensión, Chihuahua. Wilson et al. (2013a) calculated its EVS as 9, placing it at the upper limit of the low vulnerability category. The IUCN determined its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ana B. Gatica-Colima.*



No. 47. *Salvadora deserticola* Schmidt, 1940. The Big Bend Patch-nosed Snake “ranges from southeastern Arizona, southwestern New Mexico and the Big Bend in Texas southward into northern Mexico. In Mexico, this species occurs west of the Sierra Madre Occidental from eastern Sonora and adjacent southwestern Chihuahua south to northern Nayarit, and east of the Sierra Madre Occidental through most of Chihuahua” (Heimes 2016: 146). This individual was photographed in the plains near a small creek, north of Sierra El Capulín in Ascension, Chihuahua. Wilson et al. (2013a) estimated its EVS as 14, placing it at the lower limit of the high vulnerability category. The IUCN has not calculated its conservation status, and this species is not listed by SEMARNAT. *Photo by Eduardo F. Macías-Rodríguez.*



No. 48. *Diadophis punctatus* (Linnaeus, 1766). The Ring-necked Snake occurs “from southeastern Canada through the eastern and southern United States south into central Mexico” (Heimes 2016: 227). This adult was photographed among the reddish rocks characteristic of this area, where the vegetation consists of a mixture of microphyllous and rosetophyllous scrub with patches of grassland, at Cerro Colorado in the Sierra Nombre de Dios, in the municipality of Chihuahua, Chihuahua. Wilson et al. (2013a) calculated its EVS as 4, placing it in the lower portion of the low vulnerability category. The IUCN determined its status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ramón Isaac Miramontes-Cinco.*

*Sympholis lippiens**
Tantilla cucullata
Tantilla yaquia
*Thamnophis validus**
Trimorphodon wilkinsonii
*Tropidodipsas repleta**
*Urosaurus bicarinatus**

Finally, the following four turtle species have not been recorded from the NPAs in Chihuahua:

Chrysemys picta
*Gopherus evgoodei**
Rhinoclemmys pulcherrima
*Terrapene nelsoni**

Of these 61 species, 32 are non-endemics, 28 are country endemics, and one is a non-native. Naturally, it is not desirable for the non-native species to be established in the system of NPAs, but given the widespread nature of *Indotyphlops braminus* in Mexico and elsewhere, it seems likely that eventually it will be found in one or more of the NPAs in Chihuahua.

We also need to mention that four areas have been set aside voluntarily for conservation (ADVC) in Chihuahua: El Quemado with 1,896.68 ha; Reserva Ecológica Presa El Caldillo with 68.32 ha; Dulce Amparo de Aguila with 1,356.14 ha; and Potrero de la Lumbre with 5,453.20 ha (INEGI 2022). Recently, three more ADVC areas have been set aside: in Bocoyna, Ejido San Ignacio de Arareco (3,917.87 ha); Area ribereña Ejido Bocoyna (3,070.39 ha) and Ejido Panalachi (4,100.08 ha).

Conclusions and Recommendations

Conclusions

- A. Currently, the herpetofauna of Chihuahua consists of 186 species, including 35 anurans, five salamanders, 133 squamates (53 lizards and 80 snakes), and 13 turtles.
- B. The numbers of herpetofaunal species from the nine physiographic regions in Chihuahua range from 58 in the Sierras y Llanuras de Durango

- (SLD) to 128 in the Gran Meseta y Cañones Chihuahuenses (GMCC).
- C. The numbers of species shared among the nine physiographic regions range from 14 between the SPN and the GMCD to 72 between the SCN and the GMCC. The Coefficient of Biogeographic Resemblance (CBR) ranges from a low of 0.23 between the Sierras Plegadas del Norte (SPN) and the Gran Meseta y Cañones Duranguenses (GMCD) to 0.83 between the Sierras Plegadas del Norte (SPN) and the Llanuras y Sierras Volcánicas (LSV). The UPGMA dendrogram demonstrates that the closest relationships among the nine physiographic regions involve regions that are physically adjacent to one another in a series of swaths of two to three regions, generally oriented in a northwestern to southeastern direction and situated from the southwestern to the northeastern sectors of the state.
- D. The level of endemism of the herpetofauna in Chihuahua is relatively limited (62/186, or 33.3%), with 61 species comprising the country endemics and one species constituting a state endemic. The country endemics include 12 of 35 anurans (34.3%), four of five salamanders (80.0%), 17 of 53 lizards (32.1%), 23 of 80 snakes (28.8%), and five of 13 species (38.5%). A single lizard species (*Plestiodon multilineatus***) constitutes the only state endemic species.
- E. The distributional status of the 186 members of the herpetofauna of Chihuahua is as follows (in order of decreasing species numbers): non-endemics (121, 65.1%); country endemics (61, 32.8%); non-natives (three, 1.6%); and state endemics (one, 0.5%).
- F. The 121 non-endemic species are allocated to the following distributional categories (in order of decreasing species numbers): MXUS species (109, 90.1%); USCA species (six, 5.0%); MXSA species (three, 2.5%); MXCA species (two, 1.7%); and USSA (one, 0.8%).
- G. The principal environmental threats to the herpetofauna of Chihuahua are as follows:

Table 22. Summary of the distributional status of herpetofaunal species in protected areas in Chihuahua, Mexico. Total = total number of species recorded in the compendium of the listed protected areas.

Protected area	Number of species	Distributional status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
Papigochic	25	14	10	1	—
Tutuaca	24	15	8	1	—
Mohinora	39	25	14	—	—
Bassaseachic	71	64	7	—	—
Cumbres de Majalca	31	29	2	—	—
Janos	50	48	1	—	1
Médanos de Samalayuca	55	53	1	—	1
Campo Verde	20	8	11	1	—
Cañón de Santa Elena	55	51	3	—	1
Mapimí	37	34	3	—	—
Rio Bravo	22	21	—	—	1
Total	125	94	28	1	2



No. 49. *Heterodon kennerlyi* Kennicott, 1861. The Mexican Hog-nosed Snake “ranges from the western parts of Arizona, New Mexico and Texas south into Mexico. In Mexico, this species occurs in northern Sonora, Chihuahua, Durango, Coahuila, Zacatecas, Aguascalientes, San Luis Potosí, Nuevo León, and Tamaulipas” (Heimes 2016: 252). This individual was photographed on a rocky dirt road south of Cerro de la Cal, at Rancho El Uno, a Biosphere Reserve in Janos, Chihuahua. The IUCN has not determined its conservation status, and this species is not listed by SEMARNAT. *Photo by Eduardo F. Macias-Rodríguez.*



No. 50. *Thamnophis cyrtopsis* (Kennicott, 1861). The Black-necked Gartersnake “ranges from the southwestern United States through much of Mexico (but is absent in the Baja California Peninsula, coastal area and Yucatán Peninsula)” (Heimes 2016: 360). This individual was photographed in coniferous forest at San Juanito, in the municipality of Bocoyna, Chihuahua. Wilson et al. (2013a) ascertained its EVS as 7, placing it in the middle of the low vulnerability category. The IUCN judged its conservation status as Least Concern, but this species is listed as Threatened by SEMARNAT. *Photo by Daniele Gualdoni.*



No. 51. *Thamnophis eques* (Reuss, 1834). The Mexican Gartersnake “ranges continuously from Arizona southward through much of the Mexican Plateau to western Veracruz; an isolated population reported from Oaxaca” (Heimes, 2016: 364). This individual was photographed on a rock in oak forest at Cumbres de Majalca, a National Park in the state of Chihuahua, Chihuahua. Wilson et al. (2013a) determined its EVS as 8, placing it in the upper portion of the low vulnerability category. The IUCN determined its conservation status as Least Concern, but this species is considered as Threatened by SEMARNAT. *Photo by Sara G. Sáenz González.*



No. 52. *Thamnophis marcianus* (Baird and Girard, 1853). The Checkered Gartersnake “is continuously distributed across much of the southwestern United States and northern Mexico. It also occurs in several disjunct population from southern Mexico southward to northern Costa Rica” (Heimes, 2016: 372). This individual was photographed along a watering hole west of Rancho La Escondida, Nuevo Casas Grandes, Chihuahua. Wilson et al. (2013a) calculated its EVS as 10, placing it at the lower limit of the medium vulnerability category. The IUCN determined its conservation status as Least Concern, but this species is considered Threatened by SEMARNAT. *Photo by Alicia Santiesteban Martínez.*

land conversion and habitat loss; improper management of water quality and quantity; invasive species; climate change; fires and illegal logging; illegal trade; infectious diseases and parasites; on and off-road activities; mining; solid waste pollution; consumption of amphibians and reptiles; agriculture and livestock grazing; fear and/or confusion; and miscellaneous threats.

- H. The percentage of endemism in Chihuahua (33.3) falls within the values for the surrounding Mexican states of Coahuila, Durango, Sinaloa, and Sonora (28.6–52.9%).
- I. We evaluated the conservation status of the herpetofauna of Chihuahua using the SEMARNAT, IUCN, and EVS systems. As with all prior MCS studies, the SEMARNAT system was determined to be of minimal use, since only 68 of 183 native species (37.2%) have been assessed using this system. Of these 68 species, three are allocated to the Endangered (P) category, 23 to the Threatened (A) category, and 42 to the Special Protection (Pr) category. Most species (115, or 62.8%) remain unevaluated.
- J. The application of the IUCN conservation system by category and proportions of the 183 native species in Chihuahua is as follows: CR (one species; 0.5%); EN (one; 0.5%); VU (five; 2.7%); NT (six; 3.3%); LC (142; 77.6%); DD (11; 6.0%); and NE (17; 9.3%).
- K. The application of the EVS system of conservation assessment to the 183 native species of Chihuahua demonstrates that the categorial values increase from low vulnerability scores (41; 22.4%) to medium scores (78; 42.6%), and then decrease to high scores (64; 35.0%).
- L. A comparison of the IUCN and EVS conservation status categorizations demonstrates that only seven of the 64 high vulnerability species (10.9%) are placed in the three IUCN “threat categories.” At the other extreme, only 41 of the low vulnerability species (28.9%) are accounted for among the 142 LC species. As demonstrated in all other MCS studies, the correlation of the results from the application of the IUCN and EVS systems to the Chihuahua herpetofauna is relatively poor.
- M. An examination of the 170 out of 183 native species of the Chihuahuan herpetofauna that have been allocated to the IUCN DD, NE, and LC categories indicates that many of these species have been assessed improperly when compared to their respective EVS values, so we demonstrated how these 170 species should be re-assigned in the IUCN system to better indicate their prospects for survival in perpetuity.
- N. The Relative Herpetofauna Priority measure was used to determine the conservation significance of the nine regional herpetofaunas in Chihuahua. This analysis indicates the most significant herpetofauna, based on country and state endemic species numbers, is found in the

Gran Meseta y Cañones Chihuahuenses. The rank order of the remaining eight regions is as follows: Gran Meseta y Cañones Duranguenses, Sierra y Cañadas del Norte, Sierras y Llanuras de Durango, Sierras y Llanuras Tarahumaras, Bolsón de Mapimí, Llanuras y Médanos del Norte, Llanuras y Sierras Volcánicas, and Sierras Plegadas del Norte. Using the other RHP measure (number of high vulnerability species), the most significant region also is the Gran Meseta y Cañones Chihuahuenses. The rank order of the other eight regions by this measure is as follows: Sierras y Cañadas de Norte, Bolsón de Mapimí, Gran Meseta y Cañones Duranguenses, Llanuras y Médanos del Norte, Sierras y Llanuras Tarahumaras, Llanuras y Sierras Volcánicas, Sierras Plegadas del Norte, and Sierras y Llanuras de Durango.

- O. Eleven natural protected areas (NPAs) are established in Chihuahua, including two biosphere reserves, two national parks, six flora and fauna protected areas, and one national monument. All these areas have federal or national/federal status. These NPAs are distributed among all the physiographic areas in the state except for one. All but two of the NPAs are occupied by landowners, but all 11 have management programs and all but one have completed their herpetofaunal surveys.
- P. Of the state total of 186 species, 125 have been recorded in one or more of the NPAs, leaving 61 species still not recorded. These 61 species include 11 anurans, three salamanders, 43 squamates, and four turtles. One of the squamates is a non-native species, so it is not desired as a component of the NPA herpetofaunas.

Recommendations

- A. This study demonstrated that of the 186 species that comprise the Chihuahuan herpetofauna, 125 have been recorded from one or more of the 11 natural protected areas in the state. This situation is relatively desirable, but it still means that 61 species (32.8%) are not represented among these NPAs.
- B. Herpetofaunal surveys have been completed in 10 of the 11 NPAs. The number of herpetofaunal species known in each of these NPAs ranges from 20 to 71.
- C. What is known about the composition of the herpetofauna of the state’s NPAs can serve as a springboard for attempts to document the presence of the 61 species currently not reported from any of the NPAs. Such a study should be the first goal for the state’s conservation herpetologists.
- D. Once reasonably complete herpetofaunal surveys are available for the existing NPAs, then it will be possible to determine if any species from the state remain unaccounted for in these NPAs, and allow for the establishment of additional NPAs.
- E. Once the entire herpetofauna of Chihuahua is

considered protected within the NPAs, then monitoring programs can be established for the ongoing assessment of the health of the state's amphibian and reptiles.

- F. Accordingly, these steps need to be undertaken in advance of the rate at which the herpetofaunal populations in Chihuahua are threatened by human encroachment.

“To cope with the crises of biodiversity loss, climate change, overpopulation, and threats to the provision of life's essentials, far more is needed than scientific reports that are too often largely ignored. To rescue the human enterprise in the long run, requires strong action in the short run directed toward saving biodiversity and bringing the human enterprise within sustainable limits.” Chapter 13 in *Life: A Journey through Science and Politics* by Paul R. Ehrlich (2023)

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Literature Cited

- Aburto-Oropeza O, Hecht SB. 2018. Harnessing cross-border resources to confront climate change. *Environmental Science and Policy* 87: 128–132.
- Alatorre LC, Granados A, Bravo LC, Torre ME, Wiebe LC, Uc MI, González MO, Sánchez E, Rojas HL, Salas V. 2019. Agricultural furrow irrigation inefficiency in the basin of Bustillos Lagoon, Chihuahua, Mexico: geometric characteristics of agricultural plots and aquifer depletion. *Tecnología y Ciencias del Agua* 10(5): 241–281.
- Alonso-Castro AJ. 2014. Use of medicinal fauna in Mexican traditional medicine. Review. *Journal of Ethnopharmacology* 152: 53–70.
- Alva-Álvarez GI, Reyes-Hernández H, Palacio-Aponte ÁG, Núñez-López D, Muñoz-Robles C. 2018. Cambios en el paisaje ocasionados por incendios forestales en la región de Madera, Chihuahua. *Madera y Bosques* 24(3): e2431697.
- Alvarado-Díaz J, Suazo-Ortuño I, Wilson LD, Medina-Aguilar O. 2013. Patterns of physiographic distribution and conservation status of the herpetofauna of Michoacán, Mexico. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 128–170 (e71).
- Arriaga L, Espinoza JM, Aguilar C, Martínez E, Gómez L, Loa E, Coordinadores. 2000. *Regiones Terrestres Prioritarias de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, DF, México. Available: <http://www.conabio.gob.mx/conocimiento/regionalizacion/doctos/Tlistado.html> [Accessed: July 2023].
- Barragán-Vázquez MDR, Ríos Rodas L, Fucsko LA, Mata-Silva V, Rocha A, García-Padilla E, Johnson JD, Wilson LD. 2022. The herpetofauna of Tabasco, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 16(2) [General Section]: 1–61 (e315).
- Bonello D. 2019. Illegal logging in Chihuahua is now Mexico cartel territory. *Environmental Crime*. Available: <https://insightcrime.org/news/analysis/illegal-logging-chihuahua-mexico-cartel/v> [Accessed: August 2023].
- Breceda A, Arnaud-Franco A, Álvarez-Cárdenas S, Galina-Tessaro P, Montes-Sánchez J. 2009. Evaluación de la población de cerdos asilvestrados (*Sus scrofa*) y su impacto en la Reserva de la Biosfera Sierra La Laguna, Baja California Sur, México. *Tropical Conservation Science* 2(1): 173–188.
- Carbajal-Márquez RA, González-Quinonez F, Quintero-Díaz GE. 2015. Geographic distribution. *Indotyphlops braminus* (Brahminy Blindsnake). *Herpetological Review* 46(4): 573.
- Carbajal-Márquez RA, González-Saucedo ZY, Arenas-Monroy JC, Lara M. 2014. *Lithobates catesbeianus* (American Bullfrog). *Herpetological Review* 45(2): 277.
- Ceballos G, Davidson A, List R, Pacheco J, Manzano-Fisher P, Santos-Barrera G, Cruzado J. 2010. Rapid decline of a grassland system and its ecological and conservation implication. *PLoS ONE* 5(1): e8562.
- Chávez-Rodríguez A, Pinales-Munguia A, De la Garza-Aguilar R. 2007. Análisis de los estudios de disponibilidad del acuífero Laguna de Tarabillas y reevaluación de la misma mediante cuatro métodos hidrogeológicos alternativos. Thesis, Universidad Autónoma de Chihuahua, Facultad de Ingeniería. División de Investigación y Postgrado, Chihuahua, México. 45 p.
- Christensen JL, Davis DR, Jacobson ER, LaDuc TJ. 2020. Carapacial shell disease process revealed by long-term field study of the Yellow Mud Turtle, *Kinosternon flavescens*, in Texas. *Journal of Herpetology* 54(1): 1–8.
- Clarke-Crespo E, Jiménez-Vega F, González-Rojas JI, de la Mora-Covarrubias A. 2017. Multi-objective

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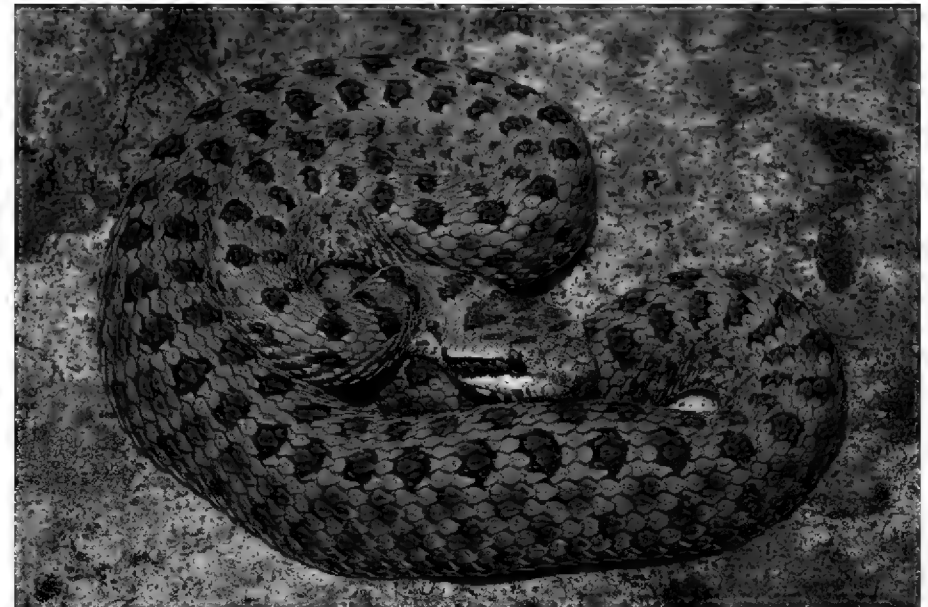
No. 53. *Crotalus atrox* Baird and Girard, 1853. The distribution of the Western Diamond-backed Rattlesnake “extends from Arkansas and north-central Oklahoma westward to southeastern California and southward through parts of Arizona, New Mexico, and much of Texas. In Mexico, this species ranges from northeastern Baja California through Sonora and northern Sinaloa, across most of Chihuahua except for the Sierra Madre Occidental, throughout Coahuila, Nuevo León, and Tamaulipas, and in the northeastern parts of Durango and Zacatecas. It also occurs in Hidalgo and Querétaro, and in parts of central and eastern San Luis Potosí, as well as in extreme northern Veracruz...” (Lemos-Espinal and Dixon 2013: 249–250). This adult was photographed in a sand dune with some vegetation, including mesquite, at Rancho Zorro Plateado, in Juárez, Chihuahua. Wilson et al. (2013a) judged its EVS as 9, placing it at the upper limit of the low vulnerability category. The IUCN determined its conservation status as Least Concern, but this species is listed as subject to Special Protection by SEMARNAT. *Photo by Jesús M. Martínez-Calderas.*



No. 54. *Crotalus lepidus* (Kennicott, 1861). The distribution of the Rock Rattlesnake “extends from southeastern Arizona, west-central and southern New Mexico, and much of southwestern Texas, in the United States, and in Mexico along the Sierra Madre Occidental from Chihuahua and Sonora southward to eastern Nayarit and Durango, and east of these mountains to the Sierra Madre Oriental in Nuevo León and Tamaulipas, and southward to western and central San Luis Potosí and westward to Zacatecas and Aguascalientes” (Lemos-Espinal and Dixon 2013: 250–251). This snake was photographed on dry leaves in pine forest at National Park Cascadas de Basaseachi, in the municipality of Ocampo, Chihuahua. Wilson et al. (2013a) determined its EVS as 12, placing it in the middle portion of medium vulnerability category. The IUCN designated its conservation status as Least Concern, but this species is listed as subject to Special Protection by SEMARNAT. *Photo by Sara G. Sáenz González.*



No. 55. *Crotalus molossus* (Baird and Girard, 1853). The distribution of the Black-tailed Rattlesnake “extends from northwestern Arizona and western New Mexico, in the United States, and in Sonora, Mexico including Isla Tiburón in the Sea of Cortés (Gulf of California), southward throughout the Sierra Madre Occidental to central Oaxaca, and throughout the Mexican plateau to southern Coahuila and Nuevo León. In San Luis Potosí it has been recorded in several localities...” (Lemos-Espinal and Dixon 2013: 252–253). This snake was photographed along a dry tree trunk in oak forest, on pieces of dry bark, at Rancho Teseachi, in the municipality of Bachiniva, Chihuahua. The IUCN determined its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Sara G. Sáenz-González.*



No. 56. *Crotalus pricei* Van Denburgh, 1895. The distribution of the Twin-spotted Rattlesnake “extends from southeastern Arizona, in the United States, southward in Mexico through the Sierra Madre Occidental in Sonora, Chihuahua, and Durango, and in the Sierra Madre Oriental, in Coahuila, Nuevo León, and Tamaulipas, with isolated populations in San Luis Potosí and Aguascalientes” (Lemos-Espinal and Dixon 2013: 254–255). This snake was photographed on a rock in pine forest at Monterde, in the municipality of Guazapares, Chihuahua. The IUCN assessed its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Eric Centenero-Alcalá.*

- method to assess the quality of grasslands in the northern Chihuahuan Desert. *Arido-Ciencia* 2017: 24–35.
- Comisión Nacional Forestal CONAFOR. 2010. *Incendios Forestales. Guía Práctica para Comunicadores*. CONAFOR, México, DF, México. 54 p.
- CONABIO; SEDUE, Gobierno de Chihuahua. 2015. *Estrategia para la Conservación y el Uso Sustentable de la Biodiversidad del Estado de Chihuahua*. Gobierno de Chihuahua, Chihuahua, México. 149 p.
- CONABIO. 2017. *Evaluación rápida de invasividad de Cyprinus carpio. Sistema de información sobre especies invasoras en México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México DF, México. 11 p.
- CONAGUA. 2020. Registro Público de Derechos de Agua (REPDa). Available: <http://sina.conagua.gob.mx/sina/tema.php?tema=usosAgua&ver=reporte&o=0&n=nacional> [Accessed: July 2023].
- Conant R, Collins JT. 1991. *A Field Guide to Reptiles and Amphibians: Eastern and Central North America*. Third Edition. Houghton Mifflin, Boston, Massachusetts, USA. xiv+150 p.
- Cotera M, Brenner J, Medel J. 2001. *Reporte Final de la Profundización Biológica de la Ecorregión de Desierto Chihuahuense en México*. Pronatura Noreste, Nuevo León, Tamaulipas, México.
- Cruz-Elizalde R, Ramírez-Bautista A, Pineda-López R, Mata-Silva V, DeSantis DL, García-Padilla E, Johnson JD, Rocha A, Fucsko LA, Wilson LD. 2022. The herpetofauna of Querétaro, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 16(1) [General Section]: 148–192 (e308).
- Cruz-Sáenz D, Muñoz-Nolasco FJ, Mata-Silva V, Johnson JD, García-Padilla E, Wilson LD. 2017. The herpetofauna of Jalisco, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 22–118.
- Dean WR, Seymour CL, Joseph GS, Foord SH. 2019. A review of the impacts of roads on wildlife in semi-arid regions. *Diversity* 11(5): 81.
- De Queiroz K. 2022. The correct name for the taxon ranked as a family containing the genus *Anolis* under rank-based nomenclature and the author of the name *Anolis loysiana*. *Herpetological Review* 53: 418–420.
- De Queiroz K. 2023. Authorship of the name Anolidae. *Herpetological Review* 54: 211–212.
- Díaz-Cervantes RE, Bravo-Peña LC, Alatorre-Cejudo LC, Sánchez-Flores E. 2014. Análisis geoespacial de la interacción entre el uso de suelo y de agua en el área peri-urbana de Cuauhtémoc, Chihuahua. Un estudio socioambiental en el norte de México. *Investigaciones Geográficas, Boletín del Instituto de Geografía UNAM* 83: 116–130.
- Diario Oficial de la Federación. 2002. *Declaración de Protección a la Denominación de Origen Sotol*. Available: <https://www.gob.mx/cms/uploads/attachment/file/494502/DO.Sotol.08.08.2002.pdf> [Accessed: August 2023].
- Dong-Min H, Ding-Qi R. 2022. Microplastics: their effects on amphibians and reptiles-a review. *Pakistan Journal of Zoology* 54(6): 2,931–2,951.
- Ehrlich PR. 2023. *Life: a Journey through Science and Politics*. Yale University Press, New Haven, Connecticut, USA. xxi+374 p.
- Estrada CAE, Spellenberg R, Lebgue T. 1997. Flora vascular de la Laguna de Babícara, Chihuahua, México. *SIDA, Contributions to Botany* 17(4): 809–827.
- Estrada-Castillón E, Jurado E, Navar JJ, Jiménez-Pérez J, Garza-Ocañas F. 2003. Plant associations of Cumbres de Majalca National Park, Chihuahua, Mexico. *The Southwestern Naturalist* 48(2): 177–187.
- Estrada-Castillón E, Villarreal-Quintanilla JA. 2010. Flora del centro del estado de Chihuahua, México. *Acta Botánica Mexicana* 92: 51–118.
- Fernández-López A, Lavín-Murcio PA. 2016. Riqueza y diversidad de anfibios y reptiles en un gradiente altitudinal en la Sierra de Juárez, Chihuahua, México. *Acta Zoológica Mexicana (Nueva Serie)* 32(3): 230–239.
- Fitzgerald LA, Painter CW, Reuter A, Hoover C. 2004. *Collection, Trade, and Regulation of Reptiles and Amphibians of the Chihuahuan Desert Ecoregion. TRAFFIC North America*. World Wildlife Fund, Washington, DC, USA. 113 p.
- Frost DR. 2023. Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <https://amphibiansoftheworld.amnh.org> [Accessed: 30 November 2023].
- Gallo-Reynoso JP, Barra-Acuña ID, Farías-González V, Macías-Sánchez S, Armenta-Méndez L, Ponce-García G, Guerrero-Flores JJ, Brito-Rios JGA, Quintana-Salvador TE, Ortega-Padilla AA. 2020. New records of *Castor canadensis* from the Conchos River, Chihuahua, México. *Therya Notes* 1(1): 115–119.
- García-García SA, Narváez-Flores R, Olivas-García JM, Hernández-Salas J. 2019. Diversidad y estructura vertical del bosque de pino-encino en Guadalupe y Calvo, Chihuahua. *Revista Mexicana de Ciencias Forestales* 10(53): 41–63.
- Garza-Almanza V, Cervantes-Rendón E, Figueroa-Parra I, Garza-Sánchez B. 2010. Rutas de tráfico ilegal de vida silvestre en Chihuahua. *CULCyT* 7(36): 5–9.
- Gatica-Colima A, Bojórquez-Rangel G. 1999. Comercio ilegal de fauna silvestre en Ciudad Juárez, Chihuahua, México. Pp. 32–33 In: *Fifth Symposium on Resources of the Chihuahuan Desert Region: U.S. and Mexico*. Sul Ross State University, Alpine, Texas, USA. 36 p.
- Gatica-Colima A, Aguirre-Terrones A, Muñoz-Rivas C. 2009. *Hemidactylus turcicus* (Mediterranean Gecko). *Herpetological Review* 40(4): 452.
- Gatica-Colima A, Jiménez-Castro JA. 2009. Serpientes de cascabel: percepción por algunos pobladores del desierto chihuahuense en el estado Chihuahua.

- Revista Latinoamericana de Recursos Naturales* 5(3): 198–204.
- Gatica-Colima A, Macias-Rodríguez E, Paredes-León R. 2014c. *Crotalus viridis viridis* (Prairie Rattlesnake). Ectoparasites. *Herpetological Review* 45(1): 143–144.
- Gatica-Colima A, Muñoz-Rivas C, Aguirre-Terrones A, Martínez-Calderas J. 2014b. *Lithobates catesbeianus* (American Bullfrog). *Herpetological Review* 45(2): 277.
- Gatica-Colima A, Navarrete-Laborde B, Ortiz-González A, Rosas-Rosas OC. 2014a. Nuevo registro de distribución del puerco espín del norte *Erethizon dorsatum* en Chihuahua, México. *Acta Zoológica Mexicana (Nueva Serie)* 30(2): 399–402.
- Gatica-Colima A, Robles-Hernández A, Rivera-Hernández A, Torres-Durán A. 2016. *Phrynosoma cornutum* (Texas Horned Lizard). Mortality. *Herpetological Review* 47(2): 301.
- Gatica-Colima A, Torres-Durán A, Martínez-Calderas J. 2017. *Trachemys gaigeae* (Mexican Plateau Slider). *Herpetological Review* 48(2): 387.
- Gavilán García IC, Fernández Villagómez G, Menchaca Pérez A, Barraza Torres LA, Gavilán García A. 2017. Policy proposal for metals speciation in tailing contaminated soils: a case study in Chihuahua, Mexico. *Journal of the Mexican Chemical Society* 61(1): 14–22.
- Global Invasive Species Database. 2013. *Cyprinus carpio*. Available: <http://www.iucngisd.org/gisd/species.php?sc=60> [Accessed: July 2023].
- Goldberg SR, Bursey CR. 1991. Helminths of three toads, *Bufo alvarius*, *Bufo cognatus* (Bufonidae), and *Scaphiopus couchii* (Pelobatidae), from southern Arizona. *Journal of the Helminthological Society of Washington* 58(1): 142–146.
- González-Elizondo MS, González-Elizondo M, Márquez-Linares MA. 2007. *Vegetación y Ecorregiones de Durango*. Plaza y Valdés Editores-Instituto Politécnico Nacional, Durango, México. 167 p.
- González PA, Sosa CM. 2003. Análisis de la vegetación del área de protección de flora y fauna Cañón de Santa Elena (desierto chihuahuense, México) utilizado Modelos Digitales de Elevación. *Ecosistemas* 12(2): 1–6.
- González-Sánchez VH, Johnson JD, García-Padilla E, Mata Silva V, DeSantis DL, Wilson LD. 2017. The herpetofauna of the Mexican Yucatan Peninsula: composition, distribution, and conservation. *Mesoamerican Herpetology* 4: 263–380.
- González-Sánchez VH, Johnson JD, González-Solís D, Fucsko LA, Wilson LD. 2021. A review of the introduced herpetofauna of Mexico and Central America, with comments on the effects of invasive species and biosecurity methodology. *ZooKeys* 1022: 79–154.
- Guerrero MT, Reed C, Vegter B. 2000. The forestry in the Sierra Madre of Chihuahua: social, economic, and ecological impacts. De los Derechos Humanos AC, Chihuahua City, Chihuahua, Mexico, and the Texas Center for Policy Studies, Austin, Texas, USA. Available: <http://www.texascenter.org/publications/forestry.pdf> [Accessed: August 2023].
- Gutiérrez-Ruiz M, Romero FM, Gonzalez-Hernandez G. 2007. Soils and sediments affected by the dispersion of metal sulfide from inactive tailings in the Santa Barbara mining area, Chihuahua, Mexico. *Revista Mexicana de Ciencias Geológicas* 24(2): 170–184.
- Gutiérrez R, Rubio-Arias H, Quintana R, Ortega JA, Gutiérrez M. 2008. Heavy metals in water of the San Pedro River in Chihuahua, Mexico, and its potential health risk. *International Journal of Environmental Research and Public Health* 5(2): 91–98.
- Gutiérrez M, Alarcón-Herrera MT, Camacho LM. 2009. Geographical distribution of arsenic in sediments within the Rio Conchos Basin, Mexico. *Environmental Geology* 57: 929–935.
- Guzmán-Martínez F, Arranz-González JC, Tapia-Téllez A. 2023. Assessment of potential contamination and acid drainage generation in uranium mining zones of Peña Blanca, Chihuahua, Mexico. *Environmental Monitoring and Assessment* 195: 386.
- Hans-Werner H, Porarowski KM, Ochoa A, Schuett GW. 2017. An interstate highway affects gene flow in a top reptilian predator (*Crotalus atrox*) of the Sonoran Desert. *Conservation Genetics* 18: 911–924.
- Heimes P. 2016. *Herpetofauna Mexicana Volume 1. Snakes of Mexico*. Edition Chimaira, Frankfurt am Main, Germany. 572 p.
- Hernández-Martínez LA, Romero-Méndez U, González-Barrios JL, García-De la Peña MC, Amézquita-Torres A. 2019. Nuevos registros y prevalencia de *Batrachochytrium dendrobatidis* en anuros de la cuenca Nazas-Aguanaval en la región norte-centro de México. *Revista Mexicana de Biodiversidad* 90: e902934.
- Instituto Nacional de Estadística y Geografía INEGI. 1999. *Estudio Hidrológico del Estado de Chihuahua*. INEGI, Aguascalientes, Aguascalientes, México. 222 p.
- Instituto Nacional de Estadística y Geografía INEGI. 2003. *Síntesis de Información Geográfica de Chihuahua*. INEGI. Aguascalientes, Aguascalientes, México. 156 p.
- Instituto Nacional de Estadística y Geografía INEGI. 2021a. *Censo de Población y Vivienda (2020). Panorama Sociodemográfico de México, Censo de Población y Vivienda*. INEGI. México, DF, México. 101 p.
- Instituto Nacional de Estadística y Geografía INEGI. 2021b. *Anuario Estadístico y Geográfico por Entidad Federativa 2020*. INEGI. México, DF, México. 626 p.
- Instituto Nacional de Estadística y Geografía INEGI. 2021c. *Cuentas de los Ecosistemas de México. Resultados del Proyecto Natural Capital Accounting and Valuation of Ecosystem Services*



No. 57. *Crotalus scutulatus* (Kennicott, 1861). The distribution of the Mohave Rattlesnake “extends from southern California, southern Nevada, and southwestern Utah southward through southern Arizona, New Mexico, and western Texas, in the United States, and in Mexico from northern Sonora and east of the Sierra Madre Occidental to Puebla and central Veracruz. The entire Chihuahuan Desert is a major portion of its distribution, especially western of Nuevo León and Tamaulipas. In San Luis Potosí it has been recorded in the west-central parts of the state” (Lemos-Espinal and Dixon 2013: 254–255). This individual was photographed on the ground with scrub vegetation, south of Sierra El Capulín in Ascensión, Chihuahua. The IUCN evaluated its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Eduardo F. Macias-Rodríguez.*



No. 58. *Crotalus viridis* (Rafinesque, 1818). The Prairie Rattlesnake “ranges from Alberta, Canada, southward over much of the Great Plains southward to northern Mexico. In Mexico, this species is known from extreme northwestern Chihuahua and northern Coahuila and has been reported from extreme northeastern Sonora...” (Heimes 2016: 487). This individual was photographed on sandy soil at Rancho Zorro Plateado in the Natural Protected Area Médanos de Samalayuca, in Juárez, Chihuahua. Wilson et al. (2013a) calculated its EVS as 12, placing it in the middle of the medium vulnerability category. The IUCN assessed its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Alejandro García-Palacios.*



No. 59. *Crotalus willardi* Meek, 1906. The Ridge-nosed Rattlesnake ranges from “extreme southeastern Arizona and southwestern New Mexico southward through the Sierra Madre Occidental and associated mountains” (Heimes 2016: 495). This individual was photographed on dry pine leaves in pine forest at Divisadero, in the municipality of Urique, Chihuahua. Wilson et al. (2013a) designated its EVS as 13, placing it at the upper limit of the medium vulnerability category. The IUCN judged its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Eric Centenero-Alcalá.*



No. 60. *Terrapene ornata* (Agassiz, 1857). The Ornate Box Turtles occupies “the central and southern Great Plains of the United States, extending southwestward into southeastern Arizona and southeastward into southwestern Louisiana. It occurs in northwestern northern and eastern Chihuahua...” (Lemos-Espinal and Smith 2007: 298–299). This individual was photographed in the grasslands of Rancho El Uno, Reserva de la Biosfera de Janos, Janos, Chihuahua. Wilson et al. (2013a) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN determined its conservation status as Near Threatened, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Eduardo F. Macias-Rodríguez.*

- (NCAVES). INEGI. México, DF, México. 258 p.
- Instituto Nacional de Estadística y Geografía INEGI. 2022. *Aspectos Geográficos. Chihuahua 2021*. INEGI, Mexico, DF, México. 8 p.
- IUCN. 2023. Red List of Threatened Species. International Union for the Conservation of Nature, Gland, Switzerland. Available: <https://www.iucnredlist.org/> [Accessed: 20 December 2023]
- Johnson JD, Mata-Silva V, García-Padilla E, Wilson LD. 2015a. The herpetofauna of Chiapas, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 2: 271–329.
- Johnson JD, Mata-Silva V, Wilson LD. 2015b. A conservation reassessment of the Central American herpetofauna based on the EVS measure. *Amphibian & Reptile Conservation* 9 [General Section]: 1–94 (e100).
- Johnson JD, Wilson LD, Mata-Silva V, García-Padilla E, DeSantis D. 2017. The endemic herpetofauna of Mexico: organisms of global significance in severe peril. *Mesoamerican Herpetology* 4: 543–620.
- Kolbert E. 2014. *The Sixth Extinction*. Picador, Henry Holt and Company, New York, New York, USA. 319 p.
- Lara-Reséndiz RA, Gadsden H, Rosen PC, Sinervo B, Méndez-De la Cruz FR. 2015. Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. *Journal of Thermal Biology* 48: 1–10.
- Lavín-Murcio PA, Arroyo-Rageb E, Quiñónez-Martínez M. 2014. Plan de acción para la conservación de los anfibios y reptiles de Chihuahua tales como el ajolote tarahumara (*Ambystoma rosaceum*), la rana ladadora tarahumara (*Craugastor tarahumaraensis*), la serpiente de cascabel de la pradera (*Crotalus viridis*) y la tortuga del Desierto Chihuahuense (*Gopherus flavomarginatus*). Pp. 84–87 In: De la Maza-Benignos M, Editor. *Plan de Acción para la Conservación y Recuperación de Especies de Fauna Silvestre Prioritaria en el Estado de Chihuahua*. Pronatura Noreste, A.C. y Gobierno del Estado de Chihuahua, Chihuahua, México. 140 p.
- Lazcano D, Salinas-Camarena MA, Contreras-Lozano JA. 2009a. Herpetological notes on the northeast of Mexico 12: Are DORs taking their toll in the snake populations? *Bulletin of the Chicago Herpetological Society* 44(5): 69–75.
- Lazcano D, Farr WL, Lavín-Murcio PA, Contreras-Lozano JA, Kardon A, Narváez-Torres S, Chávez-Cisneros JA. 2009b. Notes on Mexican herpetofauna 13: DORs in the municipality of Aldama Tamaulipas, Mexico. *Bulletin of the Chicago Herpetological Society* 44(12): 181–195.
- Lazcano D, Esquivel-Arévalo DB, Heredia-Villarreal AI, Navarro-Velázquez B, Nevárez de los Reyes M. 2017. Notes on Mexican herptofauna 31: Are roads in Nuevo León, Mexico, taking their toll on snake populations II? *Bulletin of the Chicago Herpetological Society* 52(11): 185–194.
- Lazcano D, Lavín-Murcio P, Peña-Avilés K, Quiñónez-Martínez M, Fucsko LA, Wilson LD. 2023. Notes on the herpetofauna of Mexico 42: An incident of hail killing a *Crotalus viridis* in Chihuahua, México. *Bulletin of the Chicago Herpetological Society* 58(5): 65–68.
- Lazcano D, Nevárez-de los Reyes M, García-Padilla E, Johnson JD, Mata-Silva V, DeSantis DL, Wilson LD. 2019. The herpetofauna of Coahuila, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 13(2) [General Section]: 31–94 (e189).
- Lebgue T, Sosa M, Soto R. 2005. La flora de las Barrancas del Cobre, Chihuahua, México. *Ecología Aplicada* 4(1–2): 17–23.
- Legler JM, Vogt RC. 2013. *The Turtles of Mexico: Land and Freshwater Forms*. University of California Press, Berkeley, California, USA. xi+402 p.
- Lemos-Espinal JA, Dixon JR. 2013. *Amphibians and Reptiles of San Luis Potosí*. Eagle Mountain Publishing, Eagle Mountain, Utah, USA. xii+300 p.
- Lemos-Espinal JA, Smith HM, Cruz A. 2013. *Amphibians & Reptiles of the Sierra Tarahumara of Chihuahua, Mexico*. ECO Herpetological Publishing & Distribution, Rodeo, New Mexico, USA. viii+405 p.
- Lemos-Espinal JA, Smith GR, Gadsden-Esparza H, Valdez-Lares R, Woolrich-Piña GA. 2018a. Amphibians and reptiles of the state of Durango, Mexico, with comparisons with adjoining states. *ZooKeys* 748: 65–87.
- Lemos-Espinal JA, Smith GR, Cruz A. 2018b. *Amphibians & Reptiles of Nuevo León*. ECO Herpetological Publishing & Distribution, Rodeo, New Mexico, USA. x+370 p.
- Lemos-Espinal JA, Smith GR, Rorabaugh JC. 2019a. A conservation checklist of the amphibians and reptiles of Sonora, Mexico, with updated species lists. *ZooKeys* 829: 131–160.
- Lemos-Espinal JA, Smith GR, Valdez Lares R. 2019b. *Amphibians and Reptiles of Durango, Mexico*. ECO Herpetological Publishing & Distribution, Rodeo, New Mexico, USA. xii+416 p.
- Lemos-Espinal JA, Smith GR. 2020. A checklist of the amphibians and reptiles of Sinaloa, Mexico with a conservation status summary and comparisons with neighboring states. *ZooKeys* 931: 85–114.
- Lemos-Espinal JA, Smith HM. 2007. *Anfibios y Reptiles del Estado de Chihuahua, México*. UNAM, CONABIO, México, DF, México. xiii+613 p.
- Leyte-Manrique A, Mata-Silva V, Báez-Montes O, Fucsko LA, DeSantis DL, García-Padilla E, Rocha A, Johnson JD, Porras LW, Wilson LD. 2022. The herpetofauna of Guanajuato, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 16(2) [General Section]: 133–180 (e321).
- Loredo-Varela JL, Hernández-Escudero L. 2021. Presencia del Rascón Cara Gris (*Rallus limicola*) en el sitio Ramsar Río San Pedro-Meoqui, Chihuahua, México. *Huitzil Revista Mexicana de Ornitología* 22(2): e-620.

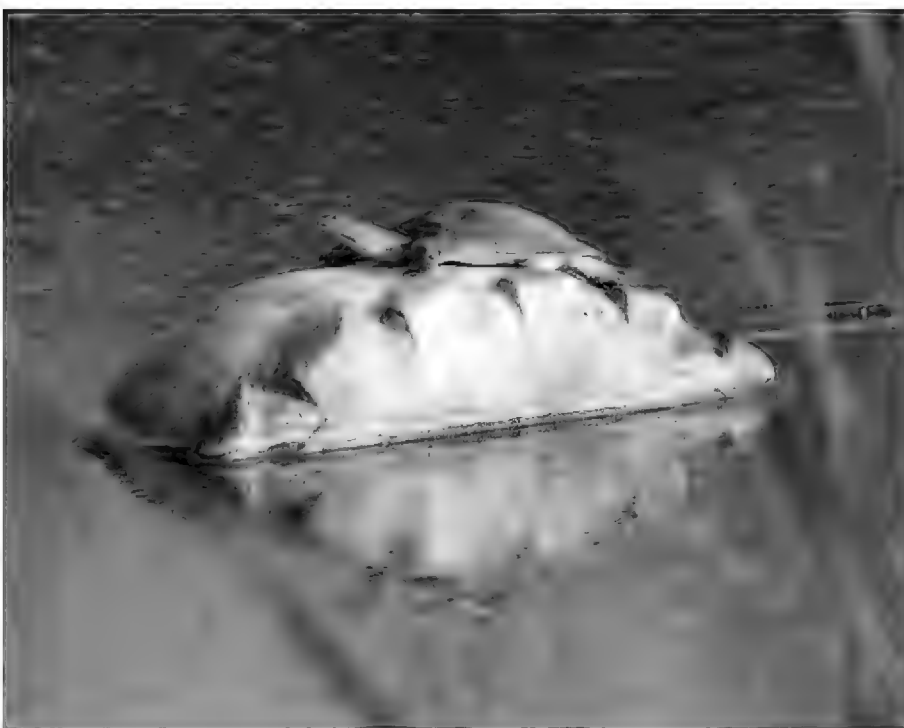
- Macías-Duarte A, Montoya AB, Hunt WG, Lafón-Terrazas A, Tafanelli R. 2004. Reproduction, prey, and habitat of the Aplomado Falcon (*Falco femoralis*) in desert grasslands of Chihuahua, Mexico. *The Auk* 121(4): 1,081–1,093.
- Martínez MJ. 1991. Flora y fitogeografía de la vegetación alpina y subalpina del Cerro Mohinora, Sierra Madre Occidental, Chihuahua, México. *Investigación y Ciencia* 3: 26–28.
- Mason JR, Latella IA, Giermakowski JT, Snell H, Poe S, Pangle RE, Gehres N, Pockman WT, McDowell NG. 2016. Too dry for lizards: short-term rainfall influence on lizard microhabitat use in an experimental rainfall manipulation within a piñon-juniper. *Functional Ecology* 30: 964–973.
- Mata-Silva V, Johnson JD, Wilson LD, García-Padilla E. 2015. The herpetofauna of Oaxaca, Mexico: composition, physiographic distribution, and conservation. *Mesoamerican Herpetology* 2: 5–62.
- Mata-Silva V, García-Padilla E, Rocha A, DeSantis DL, Johnson JD, Ramírez-Batista A, Wilson LD. 2021. A reexamination of the herpetofauna of Oaxaca, Mexico: composition update, physiographic distribution, and conservation commentary. *Zootaxa* 4996: 201–252.
- Mayani-Parás F, Botello F, Castañeda S, Sánchez-Cordero V. 2019. Impact of habitat loss and mining on the distribution of endemic species of amphibians and reptiles in Mexico. *Diversity* 210: 2–11.
- McCranie JR, Wilson LD. 1987. The biogeography of the herpetofauna of the pine-oak woodlands of the Sierra Madre Occidental of México. *Milwaukee Public Museum Contributions in Biology and Geology* 72: 1–30.
- Mendoza-Almeralla C, Burrowes P, Parra-Olea G. 2015. La quitridiomycosis en los anfibios de México: una revisión. *Revista Mexicana de Biodiversidad* 86: 238–248.
- Morafka D. 1977. *A Biogeographical Analysis of the Chihuahuan Desert through Its Herpetofauna*. University of Southern California, Los Angeles, California, USA. 313 p.
- Moreno-Contreras I, Moncada-Fernández F, Sánchez-González LA, Navarro-Sigüenza AG. 2021. An isolated population of the secretive, endemic Aztec Rail (*Rallus tenuirostris*) in Chihuahua, Mexico. *The Wilson Journal of Ornithology* 133(3): 417–425.
- Munguia-Vega A, Rodríguez-Estrella E, Shaw WW, Culver M. 2013. Localized extinction of an arboreal desert lizard caused by habitat fragmentation. *Biological Conservation* 157 (2013): 11–20.
- Nevárez de los Reyes M, Lazcano D, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD. 2016. The herpetofauna of Nuevo León, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 3: 557–638.
- Peralta-García A, Valdez-Villavicencio JH, Fucsko LA, Hollingsworth BD, Johnson JD, Mata-Silva V, Rocha A, DeSantis DL, Porras LW, Wilson LD. 2023. The herpetofauna of the Baja California Peninsula and its adjacent islands, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 17 (1&2) [General Section]: 57–142 (e326).
- Pérez-Espejo R. 2008. El lado oscuro de la ganadería. Problemas del desarrollo. *Revista Latinoamericana de Economía* 39(154): 217–227.
- Pérez-Ramos E, Luja-Molina H. 2022. Dos especies nuevas de ranas leopardo del género *Rana* (Anura: Ranidae) en la vertiente del Pacífico, al noroeste de México. *Revista de Zoología* 34: 19–41.
- Pineda-Martínez LF, León-Cruz JF, Carbajal N. 2020. Analysis of severe storms and tornado formation in the northern region of Mexico. *Revista Bio Ciencias* 7: e885.
- PMARP. 2012. *Plan Maestro de la Alianza Regional para la Conservación de los Pastizales del Desierto Chihuahuense 2011–2016*. Guzmán-Aranda JC, Hoth J, Berlanga H, Editors. Comisión para la Cooperación Ambiental, Montreal, Canada. 64 p.
- Pool DB, Panjabi AO, Macías-Duarte A, Solhjem DM. 2014. Rapid expansion of croplands in Chihuahua, Mexico, threatens declining North American grassland bird species. *Biological Conservation* 170: 274–281.
- Quiñónez-Martínez M, Enríquez-Anchondo ID, Flores-Margez JP, Palacios-Ramírez KY, Olivas-Sánchez MP, Garza-Ocañas F, Lebgue-Keleng T, Nájera-Medellín JA. 2018. Plant communities in soil of semiarid ecosystem and their relationship with mycorrhizal fungi. *Terra Latinoamericana* 36(4): 381–391.
- Ramírez-Bautista A, Hernández-Salinas U, Cruz-Elizalde R, Berriozabal-Islas C, Moreno-Lara I, DeSantis DL, Johnson JD, García-Padilla E, Mata-Silva V, Wilson LD. 2020. The herpetofauna of Hidalgo, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 14(1) [General Section]: 63–118 (e224).
- Ramos-Guerra S, Gatica-Colima A. 2014. Ecología alimentaria de la rana toro *Lithobates catesbeianus* (Shaw, 1802) en el noroeste de Chihuahua, México. Pp. 411–429 In: Low-Pfeng AM, Quijón PA, Peters-Recagno EM, Editors. *Especies Invasoras Acuáticas. Casos de Estudio en Ecosistemas de México*. SEMARNAT, INECC-SEMARNAT y UPEI, México, DF, México. 660 p.
- Rand McNally. 1998. *Rand McNally Road Atlas*. Rand McNally, Chicago, Illinois, USA. 127 p.
- Rentería-Villalobos M, Hanson RT, Eastoe C. 2022. Evaluation of climate variability on sustainability for transboundary water supply in Chihuahua, México. *Journal of Hydrology: Regional Studies* 44: 101207.
- Reyes-Gómez VM, Valero-Padilla D. 2014. Geografía y fisiografía. Pp. 20–25 In: *Geografía y Fisiografía en La biodiversidad en Chihuahua: Estudio de Estado*. CONABIO. México, DF, México. 561 p.
- Reyes-Gómez VM, Maganda C, Gutiérrez M, Alarcón-Herrera MT, Núñez-López D, Escolero O, Fuentes-



No. 61. *Trachemys gaigeae* (Hartweg, 1939). The Big Bend Slider occurs “in the Río Bravo in central New Mexico southward to (but not including) Coahuila, and in the Río Conchos, as well as in lakes and permanent ponds adjacent to the rivers” (Lemos-Espinal and Smith, 2007: 299–300). Once it was captured, this individual was photographed in a stream in the municipality of Coronado, Chihuahua. Wilson et al. (2013a) calculated its EVS as 18, placing it in the upper portion of the high vulnerability category. The IUCN judged its conservation status as Vulnerable, but this species is not listed by SEMARNAT. *Photo by Ana B. Gatica-Colima.*



No. 62. *Kinosternon flavescens* (Agassiz, 1857). The Yellow Mud Turtle is distributed in “the Mississippi and other Gulf drainages southward from NE Nebraska through Kansas, Oklahoma, Texas, and extreme southeastern Arizona to northern and Gulf Coastal Mexico. Isolated population occur in the northern part of the range in the United States” (Legler and Vogt 2013). This individual was photographed in a small rain pond in September, near El Bosque, Rancho El Uno, at Reserva de la Biosfera de Janos, in the municipality of Janos, Chihuahua. Wilson et al. (2013a) determined its EVS as 12, placing it in the middle of the medium vulnerability category. The IUCN evaluated its conservation status as Least Concern, and this species is not listed by SEMARNAT. *Photo by Ana Gatica-Colima.*



No. 63. *Apalone spiniferus* (Lesueur, 1827). The distribution of the Spiny Soft-shelled Turtle “extends from southeastern Canada and throughout much of the eastern and central United States, with scattered introduced and relictual populations in the western part of the country. In Mexico, this species occurs in Baja California and Sonora (introduced populations), along the main rivers from Chihuahua to Tamaulipas, and in San Luis Potosí; a relictual population also occurs in the Río Balsas of Guerrero” (Lemos-Espinal and Dixon 2013: 88–89). This individual was photographed on a discarded tire in a water channel in the municipality of Guadalupe, Chihuahua. Wilson et al. (2013a) designated its EVS as 15, placing it in the lower portion of the high vulnerability category. The IUCN determined its conservation status as Least Concern, and this species is listed as subject to Special Protection by SEMARNAT. *Photo by Ana Gatica-Colima.*

- Hernández HA, Ramos-Leal JA, Ochoa-Rivero JM. 2020. Diagnóstico sobre desarrollo sostenible en acuíferos del desierto chihuahuense en México: casos de gobernanza hídrica, nivel piezométrico, calidad de agua y cambio de uso de suelo. Pp. 39–65 In: Chávez-Ortiz GE, Cano-Aguilar, Rojas-González, Coordinadores. *Sociedad, Ambiente y Cultura. Diversas Miradas desde el Gran Desierto Chihuahuense*. Red Multidisciplinaria de Estudios del Desierto, Ciudad Juárez, Chihuahua, México. 236 p.
- Richardson K, Steffen W, Lucht W, Bendtsen J, Cornell SE, Donges JF, Druke M, Fetzer I, Bala G, von Bloh W, et al. 2023. Earth beyond six of nine planetary boundaries. *Science Advances* 9: 37.
- Ríos-Arana JV, Walsh EJ, Ortiz M. 2007. Interaction effects of multi-metal solutions (As, Cr, Cu, Ni, Pb, and Zn) on life history traits in the rotifer *Platyonus patulus*. *Journal of Environmental Science and Health* 42(A): 1,473–1,481.
- Rzedowski J. 2006. *Vegetación de México. 1ª Edición Digital*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, DF, México. 504 p.
- Santoyo-Brito E, Lemos-Espinal JA. 2010. Reparto de recursos de los gremios de lagartijas en el Cañón de Chínipas, Chihuahua, México. *Acta Zoológica Mexicana (Nueva Serie)* 26(2): 435–450.
- Sasaki K, Lesbarrères D, Watson G, Litzgus J. 2015. Mining-caused changes to habitat structure affect amphibian and reptile population ecology more than metal pollution. *Ecological Applications* 25(8): 2,240–2,254.
- Saucedo-Sánchez de Tagle ER. 2007. Notas y reflexiones etnográficas en torno a la fauna y su relación con la región celeste del cosmos rarámuri. *Cuicuilco* 14(39): 79–98.
- SCBD (Secretariat of the Convention of Biological Diversity). 2008. *Biodiversity and Agriculture: Safeguarding Biodiversity and Securing Food for the World*. Convention on Biological Diversity, United Nations Environmental Programme, Montreal, Quebec, Canada. 56 p.
- SEMARNAT (Secretaría De Medio Ambiente y Recursos Naturales). 2010. Norma Oficial Mexicana nom-059- semarnat-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. *Diario Oficial de la Federación*, 30 de diciembre de 2010, México, DF, 20 December 2023.
- SEMARNAT (Secretaría De Medio Ambiente y Recursos Naturales). 2019. Modificación del Anexo Normativo III, Lista de especies en riesgo de la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. *Diario Oficial de la Federación*, 14 de noviembre de 2019, México, DF, Mexico.
- Siliceo-Centero HH. 2021. Fauna en el Antropoceno: el caso de los anfibios y reptiles. Available: <https://scme.mx/fauna-en-el-antropoceno-el-caso-de-los-anfibios-y-reptiles/> [Accessed: August 2023].
- Solórzano LA. 2022. *Serpientes de Costa Rica: Distribución, Taxonomía e Historia Natural*. Editorial, San José, Costa Rica. 1,116 p.
- Soto-Cruz RA, Lebgue-Keleng T, Espinoza-Prieto JR, Quintana-Martínez RM, Quintana-Martínez G, Balderrama S, Zamudio-Mondragón FR, Quintana-Chávez MA, Mondaca-Fernández F. 2014. Primer registro de la Cotorra Argentina (*Myiopsitta monachus*) en Chihuahua, México. *Huitzil* 15(1): 1–5.
- Tanner WW. 1988. *Eumeces multilineatus*. *Catalogue of American Amphibians and Reptiles* 446: 1.
- Terán-Juárez SA, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD. 2016. The herpetofauna of Tamaulipas, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 3: 42–113.
- Torres-Hernández L, Ramírez-Bautista A, Cruz-Elizalde R, Hernández-Salinas U, Berriozabel-Islas C, DeSantis DL, Johnson JD, Rocha A, García-Padilla E, Mata-Silva V, et al. 2021. The herpetofauna of Veracruz, Mexico: composition, distribution, and conservation status. *Amphibian & Reptile Conservation* 15(2) [General Section]: 72–155 (e285).
- Torres-Olave ME, Uc-Campos MI, González-León MO, Bravo-Peña LC, Alatorre-Cejudo LC, Salas-Aguilar VM, Rojas-Villalobos HL, Granados-Olivas A. 2018. Aproximación espacio-temporal de *Sus scrofa* en Chihuahua México 2015. *Árido-Ciencia* 3(10): 12–20.
- United Nations Office for Disaster Risk Reduction (UNDRR). 2021. Forest fires in Mexico affect 212,000 hectares. Available: <https://gfmc.online/2021/05-2021/forest-fires-in-mexico-affect-212000-hectares.html> [Accessed: 20 December 2023]
- Vega-Mares JH, Rivero-Hernández O, Martínez-Salvador M, Melgoza-Castillo A. 2020. Análisis de la flora vascular de la Sierra Azul, Chihuahua, México. *Botanical Sciences* 98(3): 618–652.
- Wallach V. 2020a. New country and state records for *Indotyphlops braminus* (Serpentes: Typhlopidae). Part II. *Bulletin of the Chicago Herpetological Society* 55: 77–81.
- Wallach V. 2020b. First appearance of the Brahminy Blindsnake, *Virgotyphlops braminus* (Daudin 1803) (Squamata: Typhlopidae), in North America, with reference to the states of Mexico and the USA. *Reptiles & Amphibians* 27(2): 326–330.
- Wilson EO. 2002. *The Future of Life*. Alfred A. Knopf, New York, New York, USA. xxiv+229 p.
- Wilson LD, McCranie JR. 2004. The conservation status of the herpetofauna of Honduras. *Amphibian and Reptile Conservation* 3(1): 6–33 (e12).
- Wilson LD, Mata-Silva V, Johnson JD. 2013a. A conservation reassessment of the reptiles of Mexico based on the EVS measure. Contribution to Special

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- Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 1–47 (e61).
- Wilson LD, Johnson JD, Mata-Silva V. 2013b. A conservation reassessment of the amphibians of Mexico based on the EVS measure. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 97–127 (e69).
- Wilson LD, Johnson JD, Porras LW, Mata-Silva V, García-Padilla E. 2017. A system for categorizing the distribution of the Mesoamerican herpetofauna. *Mesoamerican Herpetology* 4: 901–913.
- Wilson LD, Lazcano D. 2019. Biology and society: exposing the vital linkages—the relationship between the study of life and humanity’s chances for a future. *Sociedad y Biología* 1: 19–44.
- Woolrich-Piña GA, Ramírez-Silva JP, Loc-Barragán J, Ponce Campos P, Mata-Silva V, Johnson JD, García-Padilla E, Wilson LD. 2016. The herpetofauna of Nayarit, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 3: 375–448.
- Woolrich-Piña GA, García-Padilla E, DeSantis DL, Johnson JD, Mata-Silva V, Wilson LD. 2017. The herpetofauna of Puebla, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 790–884.
- WWF. 2008. *Decisiones para Nuestro Futuro. Planes Rectores Comunitarios. Versiones Didácticas y Fichas Técnicas de Cuatro Núcleos Agrarios de la Cuenca Alta del Río Conchos*. WWF México, Benito Juárez, Ciudad de México, México. 96 p.
- Yoder HR, Gomez GW. 2007. Helminth parasite assemblages in Bullfrogs (*Rana catesbeiana*) from southeast Texas. *Texas Journal of Science* 59: 33–38.



Ana Bertha Gatica-Colima was born in Tijuana, Baja California, Mexico, and is a biologist with an M.S. in Management of Arid Zones from the Universidad Autónoma de Baja California, and a Ph.D. in Natural Resources from the Universidad Autónoma de Chihuahua. She was awarded a Diploma in Herpetology by the Sociedad Herpetológica Mexicana AC, and is a founding teacher of the Biology Program at the Universidad Autónoma de Ciudad Juárez. She is a researcher responsible for the Animal Ecology and Biodiversity Laboratory, where the scientific collection is maintained. This collection contains specimens of vertebrates mostly from the Chihuahuan Desert in the state of Chihuahua, Mexico. Ana has taught undergraduate students (in courses on chordate biology, fieldwork biology, and management of arid zones), and has been working with reptiles and amphibians for about 30 years. Her main interests are rattlesnakes of the genus *Crotalus* from the Sonoran and Chihuahuan deserts, although she also has worked with other colleagues on various aspects of vertebrate research. Ana has directed about 100 theses (undergraduate and graduate students), and has received grants for several projects, including three from CONABIO. She has participated in revising amphibian and reptile species proposals to be included, excluded, or have their categories changed in the SEMARNAT Norm. About 45 of her publications have appeared in refereed journals, and she has participated in national and international academic meetings. Ana is an active member of the Sociedad Herpetológica Mexicana AC, the Society for the Study of Amphibians and Reptiles, and the Southwestern Association of Naturalists. She also likes hiking and taking photos for Naturalista.



Louis W. Porras graduated with a degree in Biology in 1971 from what today is known as Miami-Dade College (Miami, Florida, USA). Over his career he has authored or co-authored over 60 academic publications, including the descriptions of two new species, and two taxa have been named in his honor. Louis developed an interest in herpetology at an early age in his native Costa Rica. His passion for the field led him to travel to many remote areas, including throughout the Bahamas, the United States, Mesoamerica, and parts of South America. In 1968 he worked at the Houston Zoological Gardens, and from 1982 to 1984 at Utah’s Hogle Zoo. In 1976 he attended the inaugural meeting of the International Herpetological Symposium (IHS), and later served the group as Vice-President and President. In 1993, along with Gordon W. Schuett, he helped launch the journal *Herpetological Natural History*, and for IHS’ 20th anniversary, in recognition of his contributions, three former Presidents dedicated the book *Advances in Herpetoculture* in his honor. Louis’ career in publishing began in 1995, when as a member of Canyonlands Publishing Group he helped publish *Fauna* magazine. In 2002 he founded Eagle Mountain Publishing, LC, which has published such herpetological titles as *Biology of the Vipers* (2002), *Biology of the Boas and Pythons* (2007), *Amphibians, Reptiles, and Turtles in Kansas* (2010), *Conservation of Mesoamerican Amphibians and Reptiles* (2010), and *Amphibians and Reptiles of San Luis Potosí* (2013). From 2014 to 2018 he was the Publisher and Managing Editor of the journal *Mesoamerican Herpetology*, and more recently he was the Publisher and Co-editor of the book *Advances in Coralsnake Biology: with an Emphasis on South America*.



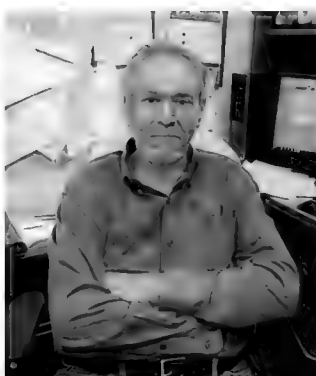
Vicente Mata-Silva is a herpetologist originally from Río Grande, Oaxaca, Mexico. His interests include the ecology, conservation, natural history, and biogeography of the herpetofaunas of Mexico, Central America, and the southwestern United States. He received his B.S. degree from the Universidad Nacional Autónoma de México (UNAM), and his M.S. and Ph.D. degrees from the University of Texas at El Paso (UTEP). Vicente is an Associate Professor of Instruction of Biological Sciences at UTEP in the Ecology and Evolutionary Biology Program, and Director of UTEP's 41,200-acre Indio Mountains Research Station, located in the Chihuahuan Desert of Trans-Pecos, Texas. To date, Vicente has authored or co-authored over 190 peer-reviewed scientific publications. He also was the Distribution Notes Section Editor for the journal *Mesoamerican Herpetology*.



Dominic L. DeSantis is an Assistant Professor of Biology at Georgia College and State University, Milledgeville, Georgia, USA, in the Department of Biological and Environmental Sciences. Dominic's research interests broadly include the behavioral ecology, conservation biology, and natural history of herpetofauna. In addition to ongoing collaborative projects associated with the Mesoamerican Research Group, much of Dominic's current research focuses on using novel animal-borne sensor technologies to study the behavior of snakes in the field. While completing his Ph.D. at the University of Texas at El Paso, Dominic accompanied Vicente Mata-Silva, Elí García-Padilla, and Larry David Wilson on survey and collecting expeditions to Oaxaca in 2015, 2016, and 2017, and is a co-author on numerous natural history publications produced from those visits, including an invited book chapter on the conservation outlook for herpetofauna in the Sierra Madre del Sur of Oaxaca.



Arturo Rocha is a Ph.D. student in the Ecology and Evolutionary Biology program at the University of Texas at El Paso. His interests include the study of biogeography, physiology, and ecology of amphibians and reptiles in the southwestern United States and Mexico. A graduate of the University of Texas at El Paso, his thesis centered on the spatial ecology of the Trans-Pecos Rat Snake (*Bogertophis subocularis*) in the northern Chihuahuan Desert. To date, he has authored or co-authored over 20 peer-reviewed scientific publications.



Jerry D. Johnson is a retired Professor of Biological Sciences at The University of Texas at El Paso, and has extensive experience studying the herpetofauna of Mesoamerica, especially that of southern Mexico. Jerry was the Director of the 41,200-acre "Indio Mountains Research Station," and was a co-editor on *Conservation of Mesoamerican Amphibians and Reptiles* and co-author of four of its chapters. He is also the senior author of the recent paper "A conservation reassessment of the Central American herpetofauna based on the EVS measure" and is Mesoamerica/Caribbean editor for the Geographic Distribution section of *Herpetological Review*. Jerry has authored or co-authored over 130 peer-reviewed papers, including two 2010 articles, "Geographic distribution and conservation of the herpetofauna of southeastern Mexico" and "Distributional patterns of the herpetofauna of Mesoamerica, a Biodiversity Hotspot." One species, *Tantilla johnsoni*, has been named in his honor. Presently, he is an Associate Editor and Co-chair of the Taxonomic Board for the journal *Mesoamerican Herpetology*.



Larry David Wilson was a renowned herpetologist with lengthy experience in Mesoamerica who passed away from leukemia on 28 April 2024. He was born in Taylorsville, Illinois, USA, and received his university education at the University of Illinois at Champaign-Urbana (B.S. degree) and at Louisiana State University in Baton Rouge (M.S. and Ph.D. degrees). He authored or co-authored 488 peer-reviewed papers and books on herpetology. Larry was the senior editor of *Conservation of Mesoamerican Amphibians and Reptiles* (2010) and a co-author of seven of its chapters. His other books include *The Snakes of Honduras* (1985), *Middle American Herpetology* (1988), *The Amphibians of Honduras* (2002), *Amphibians & Reptiles of the Bay Islands and Cayos Cochinos, Honduras* (2005), *The Amphibians and Reptiles of the Honduran Mosquitia* (2006), and *Guide to the Amphibians & Reptiles of Cusuco National Park, Honduras* (2008). He was also the co-author of 16 entries in the Mexican Conservation series, which dealt with the herpetofauna of the states of Michoacán, Oaxaca, Chiapas, Tamaulipas, Nayarit, Nuevo León, Jalisco, Puebla Coahuila, Hidalgo, Veracruz, Querétaro, Tabasco, Guanajuato, and the Baja California Peninsula, as well as the tri-state Mexican Yucatan Peninsula. In addition, he was a co-author of several significant publications on the development and extensive application of the EVS measure and on conservation issues related to the Mexican and Central American herpetofaunas. He authored or co-authored the descriptions of 76 currently recognized herpetofaunal species, and six species have been named in his honor, including the anuran *Craugastor lauraster*, the lizard *Norops wilsoni*, as well as coccidian parasite *Isospora wilsoni*. In 2005 he was designated a Distinguished Scholar in the Field of Herpetology at the Kendall Campus of Miami-Dade College by the then-campus president Dr. Wasim Shomar. Larry also served as a Co-chair of the Taxonomic Board for the website Mesoamerican Herpetology.

Appendix 1. Priority Terrestrial Regions (Regiones Terrestres Prioritarias or RTPs) found within the physiographic provinces of Chihuahua and their principal environmental problems. Numbers refer to those provided for the RTPs by CONABIO (Arriaga et al. 2000).

Sierra y Llanuras del Norte (SLN)

- Médanos de Samalayuca (48). Changes in land use (irrigation by using wells); the effects of tourism due to the use of off-road vehicles that disturb the ecological balance of the dunes; and contamination by garbage.
- Cañón de Santa Elena (49). Overgrazing, indiscriminate illegal hunting, low productivity, and high pressure on natural reserves due to the extraction of firewood and the use of fauna for food.
- El Berrendo (50). Overgrazing, desertification, and poaching.
- Laguna Jaco (51). Salt mines have highly impacted the area.
- Mapimí (52). Cattle and horse farming; the collection of the Candelilla Shrub (*Euphorbia antisiphilitica*); rain fed crops, and the effects of tourism in the Silence Zone; the extraction of salt from ephemeral lagoons, as well as minerals such as dolomite, zinc, copper, and silver; the depletion of the aquifer due to the use of water for irrigation and the use of “abrevaderos” (= watering holes); an increase in deer hunting; and growth of the urban frontier in the region of La Comarca Lagunera.
- Cuchillas de la Zarca (53). In the lower zone, rain fed crops extend into higher areas of grassland and open oak forest, which are less suitable for agriculture.

Sierra Madre Occidental (SMO)

- Guadalupe y Calvo-Mohinora (26). Certain types of crops threaten the ecological integrity of the region.
- Barranca Sinforosa (27). Illegal logging in forested areas; and the extraction and trafficking of medicinal plants, birds, and mammals.
- Rocahuachi-Nanaruchi (28). In the short term, problems occur due to highway construction and the deposition of minerals.
- Lago Los Mexicanos (29). In this region, poaching affects species with a commercial value. The lake also is being desiccated, so deforestation and changes in groundwater levels occur; the lake also is being contaminated by agrochemicals, solid waste, and urban sewage.
- Alta Tarahumara-Barrancas (30). This region is being impacted by forestry activities. For example, there has been a reduction in the number of strawberry trees (*Arbutus* sp.), as well as changes in the community structure of Lumholtz’s Pine (*Pinus lumholtzii*), locally known as “Pino Triste,” and oaks (*Quercus* sp.). This area needs restoration.

- Sierra Álamos-El Cuchujaqui (31). This area suffers from overgrazing, the excessive logging of Croton trees (*Croton* sp.), which are used for fence posts, and accelerated opening of paddocks and buffel grass prairie in the shrublands and jungles. Furthermore, birds such as the Military Macaw (*Ara militaris*), the Thick-billed Parrot (*Rhynchopsitta pachyrhyncha*), the Black-throated Magpie Jay (*Calocitta coliei*), the West-Mexican Chachalaca (*Ortalis poliocephala*), and reptiles such as the Gila Monster (*Heloderma suspectum*) and *Gopherus* sp. are collected and sold illegally.
- Cañón de Chinipas (32). The construction of a highway to Creel and a tourist project (FONATUR [= The National Fund for Tourism Development]) can lead to additional environmental problems.
- Bassaseachic (33). The clearing of land and tourist developments are affecting the higher elevations, but environmental problems also are evident in the lower elevations.
- Babicora (34). There is an accelerated process of using the “influence area” of the lagoon for agricultural activities.
- Cuenca del río Chico-Sirupa (35). Incipient progressive forest extraction that can degrade the ecosystems of the region.
- Yécora-El Reparo (36). The major environmental problems in this region are the cutting of pine forest and mining. More recently, the illegal cutting of oak trees for charcoal production has been increasing, even though it is a low-quality product.
- Bavispe-El Tigre (44). From a conservation perspective, this isolated mountain range has remained relatively untouched. Nonetheless, illegal hunting, moderate logging activities, and open-pit mining have been documented in this region.

Sierra y Llanuras del Norte (SLN) and Sierra Madre Occidental (SMO)

- Sierra San Luis-Janos (45). In this region, the introduced and invasive Buffleggrass (*Cenchrus ciliaris*) is an important environmental consideration.
- Pastizales del Norte del Río Santa María (46). Environmental problems include structural changes resulting from overgrazing in yucca, mesquite, and prickly pear shrub communities.
- Sierra del Nido-Pastizales de Flores Magón (47). Poaching and the accidental introduction of exotic wildlife are the principal threats. Deforestation is also an important environmental problem.

Addendum: Entidades federativas de México por superficie, población y densidad. Available: https://es.wikipedia.org/wiki/A:Entidades_federativas_de_M%C3%A9xico_por_superficie,_poblaci%C3%B3n_y_densidad [Accessed: 12 August 2024].



Conservation status of *Sceloporus* lizards

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Abstract.—*Sceloporus* is a diverse genus of lizards that has been widely studied regarding its evolution, behavior, and ecology. Although evidence suggests *Sceloporus* lizards are threatened by several factors, there are few studies concerning their conservation issues and status. Here we analyzed the conservation status of species of the genus *Sceloporus* based on two different systems: the IUCN Red List and the Environmental Vulnerability Scores (EVS) system. We updated the taxonomic state of the genus, investigated the conservation status of the existing species, calculated the EVS for previously unevaluated species, and generated potential distribution maps of all species based on species distribution modelling. We determined that 116 species of *Sceloporus* are currently recognized. For conservation status, we found differences between the IUCN Red List and the EVS system. According to the Red List, 64 species are in the Least Concern category, two Near Threatened, three Vulnerable, three Endangered, and one Critically Endangered (and 38 Not Evaluated); however, based on the EVS system, most of the species (69) are in the high vulnerability category, 37 in the medium category, and 10 in the low category. About half of the species in the high vulnerability category in the EVS either have not been evaluated, are deficient in data, or are of Least Concern in the IUCN Red list. Of the 116 species, we assigned 46 to conservation priority level I. Because *Sceloporus* is a widely distributed genus and there have been new cryptic species discovered, the information provided here is vital for the conservation of the genus, since it will allow us to identify *Sceloporus* species urgently in need of conservation.

Keywords. Conservation priority levels, distribution, endemic species, environmental vulnerability score, IUCN Red List, taxonomic update

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Introduction

The latest Global Assessment on Biodiversity and Ecosystem Services stated: “Nature is now declining globally at rates unprecedented in human history” (IPBES 2019). According to this assessment, approximately 1,000,000 species are threatened with extinction due to five direct drivers: 1) changes in land and sea use; 2) direct exploitation of organisms; 3) climate change; 4) pollution, and 5) invasive species (IPBES 2019). Identifying species at risk of extinction is essential for

addressing this biological crisis (Böhm et al. 2016).

Even though the extinction crisis has been explored for some groups of invertebrates (Dirzo et al. 2014) and vertebrates (Ceballos et al. 2015), reptiles as a group have received less attention and are often overlooked when it comes to conservation assessments (Gibbons et al. 2000; Todd et al. 2010; Böhm et al. 2013, 2016; Saha et al. 2018). There is also evidence that the five direct drivers of extinction act on reptile populations (see Todd et al. 2010 and Fitzgerald et al. 2018 for reviews).

Böhm et al. (2013) conducted the first global

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assessment of extinction risk for reptiles based on the IUCN Red List categories of 1,500 species. They found that 20% of the world's reptiles were threatened and 21% of the listed reptiles were in the Data Deficient category. In this context, understanding and summarizing the conservation status of reptiles is becoming a major priority (Fitzgerald et al. 2018).

Although Böhm et al. (2013) based their assessment on the IUCN Red List categorization, some other authors have questioned the usefulness of this system for assessing the conservation status of reptiles (e.g., Wilson et al. 2013; Johnson et al. 2015) and other taxa (e.g., terrestrial vertebrates, Mayani-Parés et al. 2022). Wilson et al. (2013) proposed an alternative system for evaluating the conservation status of reptiles based on three critical aspects: 1) geographic distribution, 2) ecological distribution, and 3) the degree of human persecution. Their system of EVS (Environmental Vulnerability Scores) has been widely used to evaluate the conservation status of reptiles throughout Mesoamerica (e.g., Johnson et al. 2015, 2017; Mata-Silva et al. 2019). Moreover, Johnson et al. (2017) and Mata-Silva et al. (2019) proposed the Conservation Priority status for Mesoamerican reptiles. The Conservation Priority is calculated by combining the data on the physiographic distribution and EVS group categorization of a species and considers that the smaller the number of physiographic regions occupied by a species, the more difficult its conservation will be (Johnson et al. 2017).

Sceloporus Wiegmann, 1828, is a genus of diurnal, insectivorous lizards in the family Phrynosomatidae. This genus has been the focus of several herpetological investigations, in part due to its high species diversity (up to 100+ species distributed in 18 species groups), broad geographic distribution (from northern USA to Panama), and its great ecological, morphological, and ethological diversity (Sites et al. 1992; Hall 2009; Leaché et al. 2016; Uetz et al. 2022). Evidence suggests that *Sceloporus* lizards are being threatened by several factors (Hokit and Branch 2003; Sinervo et al. 2010; Gadsden et al. 2018; Trumbo et al. 2021; Rurik et al. 2022), however, few studies have examined the conservation issues and status for the genus as a whole. Sinervo et al. (2010) predicted that about 60% of *Sceloporus* species in Mexico would be extinct by 2080 due to climate change. In addition, factors such as habitat degradation (Hokit and Branch 2003; Ernst et al. 2004; Chan et al. 2013; Gadsden et al. 2018; Walkup et al. 2018; Rurik et al. 2022) and invasive alien species (Lance et al. 2009; Thawley and Langkilde 2016; Trumbo et al. 2021) are negatively affecting populations of *Sceloporus*.

Based on the above considerations, and since there is no synthesis of the conservation status of many species in the genus *Sceloporus*, our aim was to assess the conservation status of the constituent species by employing the IUCN Red List categorizations and the Environmental Vulnerability Score (Wilson et al. 2013)

for all the species of *Sceloporus*. After a taxonomic update for the genus, we then evaluated the conservation status and developed distribution maps for each species in the genus *Sceloporus*. We also determined the conservation priority level for each species.

Materials and Methods

Taxonomic Update

The list of species of *Sceloporus* found in The Reptile Database (Uetz et al. 2022) served as a basis for the taxonomic update that followed the taxonomy proposed by Wilson et al. (2013), Leaché et al. (2016), and Johnson et al. (2017). The historic and recent literature regarding each species or species group were consulted to update the taxonomy used and identify the extant species of *Sceloporus*.

Systems for Determining Conservation Status

Both the IUCN Red List (<https://www.iucnredlist.org/>) and the EVS system (Wilson et al. 2013; Johnson et al. 2015, 2017; Mata-Silva et al. 2019; García-Padilla et al. 2020) were used to assess the conservation status of each species in the genus *Sceloporus*. The IUCN system considers seven categories of extinction risk status: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CE), Endangered (EN), Vulnerable (VU), Near Threatened (NT), and Least Concern (LC). Two other categories include taxa with insufficient information as Data Deficient (DD) and taxa that have not been evaluated by IUCN criteria as Not Evaluated (NE). Unlike the IUCN system, the EVS system does not consider the details of a species' population status. Rather, it is based on three components: 1) geographic distribution, 2) ecological distribution, and 3) degree of human persecution. The sum of the scores of these three components equals the Environmental Vulnerability Score, which ranges from 3 to 20. An EVS of 3 to 9 is considered low vulnerability to environmental degradation, while 10 to 13 indicates medium vulnerability, and 14 to 20 represents high vulnerability (Wilson et al. 2013).

EVS Calculation, and Updating and Conservation Priority Assessment of *Sceloporus* species

Since the EVS assessment was designed for Mesoamerican reptiles (Wilson and McCranie 2004; Wilson et al. 2013; Johnson et al. 2015, 2017; Mata-Silva et al. 2019), the species of *Sceloporus* endemic to the United States (US) have not been previously evaluated using the EVS criteria. Thus, we calculated the EVS for the seven species endemic to the US (*S. arenicolus*, *S. becki*, *S. consobrinus*, *S. graciosus*, *S. tristichus*, *S. undulatus*, and *S. woodi*). As mentioned above, the EVS algorithm consists of three components or scales

(Wilson et al. 2013). The first scale regarding geographic distribution was revised for use with the US *Sceloporus* species, based their occurrence data points, as follows:

- 1 = distribution broadly represented both inside and outside the US (large portions of the range are both inside and outside the US)
- 2 = distribution prevalent inside the US, but limited outside the US (most of the range is inside the US)
- 3 = distribution limited inside the US, but prevalent outside the US (most of the range is outside the US)
- 4 = distribution limited both inside and outside the US (most of the range is restricted to areas near the US-Mexico border)
- 5 = distribution only within the US, but not restricted to the vicinity of the type locality
- 6 = distribution limited to the US in the vicinity of the type locality

The second scale deals with the extent of ecological distribution and was based on the number of vegetation formations occupied according to the Ecological Regions of North America Level III (Wiken et al. 2011). The eight categories are as follows:

- 1 = occurs in eight or more formations
- 2 = occurs in seven formations
- 3 = occurs in six formations
- 4 = occurs in five formations
- 5 = occurs in four formations
- 6 = occurs in three formations
- 7 = occurs in two formations
- 8 = occurs in one formation

The third and final scale considers the degree of human persecution as proposed by Wilson et al. (2013). However, note that all *Sceloporus* species are terrestrial and generally ignored by humans (i.e., level 3):

- 1 = fossorial, usually escape human notice
- 2 = semifossorial, or nocturnal arboreal or aquatic, nonvenomous and usually non-mimicking, sometimes escape human notice
- 3 = terrestrial and/or arboreal or aquatic, generally ignored by humans
- 4 = terrestrial and/or arboreal or aquatic, thought to be harmful, might be killed on sight
- 5 = venomous species or mimics thereof, killed on sight
- 6 = commercially or non-commercially exploited for hides, meat, eggs, and/or the pet trade

As numerous taxonomic changes have occurred since Johnson et al. (2017), Mata-Silva et al. (2019), and García-Padilla et al. (2020) published their assessments, the EVS were calculated for those Mesoamerican species that have been recently described or elevated to the

species level (*S. binocularis*, *S. dixonii*, *S. geminus*, *S. hesperus*, *S. huichol*, *S. madrensis*, *S. melanogaster*, *S. mikeprestoni*, and *S. olloporus*) and the EVS of species whose distributional range changed (*S. torquatus* and *S. variabilis*) were re-evaluated. The new EVS assessments were made following the criteria of Wilson et al. (2013) and Johnson et al. (2017).

Finally, the conservation priority of each *Sceloporus* species was investigated according to Johnson et al. (2017). All the species within priority level one were obtained from Johnson et al. (2017), Mata-Silva et al. (2019), and García-Padilla et al. (2020). The conservation priority of the endemic herpetofauna of Mexico and Central America was obtained from Johnson et al. (2017) and Mata-Silva et al. (2019), respectively. The conservation priorities for the remaining species were evaluated by considering the number of physiographic regions and the EVS calculation for each species (Johnson et al. 2017).

Geographic Distribution and Endemism of *Sceloporus* Species

Species Distribution Models (SDMs) were used to obtain a more complete picture of the distributions of the *Sceloporus* species. Occurrence points were obtained from the Global Biodiversity Information Facility (GBIF) and manually cleaned. SDMs were developed using the Wallace EcoMod package for the R programming language (R Development Core Team 2015; Kass et al. 2023). The 11 climatic variables identified by Lawing et al. (2016) were used, as they are the most important for describing the modern distribution of lizards in the genus *Sceloporus*: mean diurnal range, isothermality, temperature seasonality, minimum temperature of the coldest month, temperature annual range, mean temperature of the wettest quarter, mean temperature of the driest quarter, mean temperature of coldest quarter, precipitation of the driest month, precipitation seasonality, and precipitation of coldest quarter. The set of areas accessible to each species over relevant periods of its history is termed “M,” and it is a critical determinant of the outcome of model calibration, evaluation, and comparison (Barve et al. 2011). The M was set to the Extent of Occurrence (EOO) based on raw data with a buffer of 100 km to allow for a sufficiently wide range of background localities without creating models that extend too far beyond the known distribution of the species (VanDerWal et al. 2009). Of the available data, 50% of the occurrences were used for training and 50% were used for testing. For species with between five and twenty occurrence points, their occurrences were partitioned using jackknife, which is the best method for models with few occurrences (Guisan and Zimmermann 2000). SDMs for species with fewer than five occurrences were not built. The models were limited to include only the linear and quadratic features to prevent

overfitting. Regularization multipliers were set from 0.5 to 2 in intervals of 0.5. For model selection, the average AUC, omission rate, and AICc were considered, and the statistically significant models with the lowest omission rate and delta AICc values were selected. Finally, the models were then converted to binary presence/absence maps by using the 10th percentile training presence threshold, i.e., the probability value above which 90% of the raw data will be present in the presence/absence maps (see Phillips et al. 2006). The distribution maps of each species can be found in the **Supplementary Material**. Based on the SDMs and the literature, the species of *Sceloporus* that occur in only one country (i.e., endemic species) were identified.

Results and Discussion

A Taxonomic Update

Our taxonomic update revealed that there are currently 116 species in the genus *Sceloporus* (Table 1), including some species not listed in The Reptile Database (RDB)

(Uetz et al. 2022). For example, Leaché et al. (2016) recognized *S. vandenburgianus* as an independent species and *Sceloporus prezygus* was elevated to the species level by Martínez-Méndez et al. (2012). In addition, several new *Sceloporus* species have been recently described (e.g., *S. dixonii*, Bryson et al. 2021; *S. hesperus*, Bryson et al. 2021; *S. huichol*, Flores-Villela et al. 2022; and *S. geminus*, Campillo-García et al. 2023) and others have been elevated from subspecies to species (e.g., *S. olloporus*, Solis-Zurita et al. 2019; as well as *S. binocularis*, *S. madrensis*, *S. melanogaster*, and *S. mikeprestoni*, Campillo-García et al. 2021). We also did not include *S. bimaculosus*, *S. edbelli*, and *S. scitulus*, which are subspecies not recognized as species by Leaché et al. (2016).

Conservation Status of *Sceloporus* Lizards: The IUCN vs. EVS Systems

In the current version of the IUCN Red List, 38 species of *Sceloporus* are Not Evaluated, five species are Data Deficient, 64 are Least Concern, two are Near Threatened,

Table 1. *Sceloporus* species and their conservation status levels according to IUCN Red List and the Environmental Vulnerability Score (EVS). Priority level one species are in bold. Asterisks (*) indicate species for which the EVS was re-evaluated in this work.

Species	Author	IUCN	EVS	EVS citation
<i>Sceloporus acanthinus</i>	Bocourt, 1873	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus adleri</i>	Smith and Savitzky, 1974	LC	High (15)	Wilson et al. 2013
<i>Sceloporus aeneus</i>	Wiegmann, 1828	LC	High (16)*	This study
<i>Sceloporus albiventris</i>	Smith, 1939	NE	High (16)	Wilson et al. 2013
<i>Sceloporus anahuacus</i>	Lara-Gongora, 1983	LC	High (15)	Wilson et al. 2013
<i>Sceloporus angustus</i>	(Dickerson, 1919)	LC	High (16)	Wilson et al. 2013
<i>Sceloporus arenicolus</i>	Degenhardt and Jones, 1972	V	High (15)	This study
<i>Sceloporus asper</i>	Boulenger, 1897	LC	High (14)	Wilson et al. 2013
<i>Sceloporus aurantius</i>	Grummer and Bryson, 2014	NE	High (14)	Carbajal-Márquez and Quintero-Díaz 2016
<i>Sceloporus aureolus</i>	Smith, 1942	NE	High (15)	Johnson et al. 2017
<i>Sceloporus becki</i>	Van Denburgh, 1905	NE	High (17)	This study
<i>Sceloporus bicanthalis</i>	Smith, 1937	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus binocularis</i>	Dunn, 1936	NE	High (16)	This study
<i>Sceloporus brownorum</i>	Smith, Watkins-Colwell, Lemos-Espinal, and Chiszar, 1997	NE	High (14)	Carbajal-Márquez and Quintero-Díaz 2016
<i>Sceloporus bulleri</i>	Boulenger, 1895	LC	High (15)	Wilson et al. 2013
<i>Sceloporus caeruleus</i>	Smith, 1936	NE	High (16)	Johnson et al. 2017
<i>Sceloporus carinatus</i>	Smith, 1936	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus cautus</i>	Smith, 1938	LC	High (15)	Wilson et al. 2013
<i>Sceloporus chaneyi</i>	Liner and Dixon, 1992	E	High (15)	Wilson et al. 2013
<i>Sceloporus chrysostictus</i>	Cope, 1866	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus clarkii</i>	Baird and Girard, 1852	LC	Medium (10)	Wilson et al. 2013
<i>Sceloporus consobrinus</i>	Baird and Girard, 1854	NE	Low (9)	This study
<i>Sceloporus couchii</i>	Baird, 1859	NE	High (15)	Wilson et al. 2013
<i>Sceloporus cowlesi</i>	Lowe and Norris, 1956	NE	Medium (13)	Wilson et al. 2013
<i>Sceloporus cozumelae</i>	Jones, 1927	LC	High (15)	Wilson et al. 2013
<i>Sceloporus cryptus</i>	Smith and Lynch, 1967	LC	High (14)	Wilson et al. 2013

Table 1 (continued). *Sceloporus* species and their conservation status levels according to IUCN Red List and the Environmental Vulnerability Score (EVS). Priority level one species are in bold. Asterisks (*) indicate species for which the EVS was re-evaluated in this work.

Species	Author	IUCN	EVS	EVS Citation
<i>Sceloporus cupreus</i>	Bocourt, 1873	NE	High (16)	Wilson et al. 2013
<i>Sceloporus cyanogenys</i>	Cope, 1885	NE	High (16)	Wilson et al. 2013
<i>Sceloporus cyanostictus</i>	Axtell and Axtell, 1971	E	High (16)	Wilson et al. 2013
<i>Sceloporus dixonii</i>	Bryson and Grummer, 2021	NE	High (16)	This study
<i>Sceloporus druckercolini</i>	Perez-Ramos and Saldana De La Riva, 2008	NE	High (14)	Wilson et al. 2013
<i>Sceloporus dugesii</i>	Bocourt, 1874	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus edwardtaylori</i>	Smith, 1936	LC	High (14)	Wilson et al. 2013
<i>Sceloporus esperanzae</i>	Mccranie, 2018	NE	High (14)	Mccranie 2018
<i>Sceloporus exsul</i>	Dixon, Ketchersid and Lieb, 1972	CE	High (17)	Wilson et al. 2013
<i>Sceloporus formosus</i>	Wiegmann, 1834	LC	High (15)	Wilson et al. 2013
<i>Sceloporus gadoviae</i>	Boulenger, 1905	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus gadsdeni</i>	Castañeda-Gaytán and Díaz-Cárdenas, 2017	NE	High (17)	Johnson et al. 2017
<i>Sceloporus geminus</i>	Campillo-García, Flores-Villela, Butler, Benabib, and Castiglia, 2023	NE	High (17)	This study
<i>Sceloporus goldmani</i>	Smith, 1937	E	High (15)	Wilson et al. 2013
<i>Sceloporus graciosus</i>	Baird and Girard, 1852	LC	Low (9)	This study
<i>Sceloporus grammicus</i>	Wiegmann, 1828	LC	Low (9)	Wilson et al. 2013
<i>Sceloporus grandaevus</i>	(Dickerson, 1919)	LC	High (16)	Wilson et al. 2013
<i>Sceloporus halli</i>	Dasmann and Smith, 1974	DD	High (17)	Wilson et al. 2013
<i>Sceloporus hesperus</i>	Bryson and Grummer, 2021	NE	High (17)	This study
<i>Sceloporus heterolepis</i>	Boulenger, 1895	LC	High (14)	Wilson et al. 2013
<i>Sceloporus hondurensis</i>	Mccranie, 2018	NE	Medium (13)	Mccranie 2018
<i>Sceloporus horridus</i>	Wiegmann, 1834	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus huichol</i>	Flores-Villela, Smith, Campillo-García, Martínez-Méndez, and Campbell, 2022	NE	High (16)	This study
<i>Sceloporus hunsakeri</i>	Hall and Smith, 1979	LC	High (14)	Wilson et al. 2013
<i>Sceloporus insignis</i>	Webb, 1967	LC	High (16)	Wilson et al. 2013
<i>Sceloporus internasalis</i>	Smith and Bumzahem, 1955	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus jalapae</i>	Günther, 1890	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus jarrovi</i>	Cope, 1875	NE	Medium (11)	Wilson et al. 2013
<i>Sceloporus lemosespinali</i>	Lara-Góngora, 2004	DD	High (16)	Wilson et al. 2013
<i>Sceloporus licki</i>	Van Denburgh, 1895	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus lineatulus</i>	Dickerson, 1919	LC	High (17)	Wilson et al. 2013
<i>Sceloporus lunae</i>	Bocourt, 1873	LC	High (15)	Mata-Silva et al. 2019
<i>Sceloporus lundelli</i>	Smith, 1939	LC	High (14)	Wilson et al. 2013
<i>Sceloporus macdougalli</i>	Smith and Bumzahem, 1953	LC	High (16)	Wilson et al. 2013
<i>Sceloporus maculosus</i>	Smith, 1934	V	High (16)	Wilson et al. 2013
<i>Sceloporus madreensis</i>	Olson, 1986	NE	High (17)	This study
<i>Sceloporus magister</i>	Hallowell, 1854	LC	Low (9)	Wilson et al. 2013
<i>Sceloporus malachiticus</i>	Cope, 1864	NE	Medium (10)	Mata-Silva et al. 2019
<i>Sceloporus marmoratus</i>	Hallowell, 1852	NE	Medium (11)	Wilson et al. 2013
<i>Sceloporus megalepidurus</i>	Smith, 1934	NE	High (14)	Wilson et al. 2013
<i>Sceloporus melanogaster</i>	Cope, 1885	NE	High (14)	This study
<i>Sceloporus melanorhinus</i>	Bocourt, 1876	LC	Low (9)	Wilson et al. 2013
<i>Sceloporus merriami</i>	Stejneger, 1904	LC	Medium (13)	Wilson et al. 2013

Conservation status of *Sceloporus* lizards

Table 1 (continued). *Sceloporus* species and their conservation status levels according to IUCN Red List and the Environmental Vulnerability Score (EVS). Priority level one species are in bold. Asterisks (*) indicate species for which the EVS was re-evaluated in this work.

Species	Author	IUCN	EVS	EVS Citation
<i>Sceloporus mikeprestoni</i>	Smith and Alvarez, 1974	NE	High (17)	This study
<i>Sceloporus minor</i>	Cope, 1885	LC	High (14)	Wilson et al. 2013
<i>Sceloporus mucronatus</i>	Cope, 1885	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus nelsoni</i>	Cochran, 1923	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus oregon</i>	Smith and Brown, 1941	V	High (14)	Wilson et al. 2013
<i>Sceloporus occidentalis</i>	Baird and Girard, 1852	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus ochoterenae</i>	Smith, 1934	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus olivaceus</i>	Smith, 1934	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus olloporus</i>	Smith, 1937	NE	Low (9)	García-Padilla et al. 2020
<i>Sceloporus omiltemanus</i>	Günther, 1890	NE	High (16)	Johnson et al. 2017
<i>Sceloporus orcutti</i>	Stejneger, 1893	LC	Low (7)	Wilson et al. 2013
<i>Sceloporus ornatus</i>	Baird, 1859	NT	High (16)	Wilson et al. 2013
<i>Sceloporus palaciosi</i>	Lara-Gongora, 1983	LC	High (15)	Wilson et al. 2013
<i>Sceloporus parvus</i>	Smith, 1934	LC	High (15)	Wilson et al. 2013
<i>Sceloporus poinsettii</i>	Baird and Girard, 1852	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus prezygus</i>	Smith, 1939	NE	High (15)	Wilson et al. 2013
<i>Sceloporus pyrocephalus</i>	Cope, 1864	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus salvini</i>	Günther, 1890	DD	High (15)	Wilson et al. 2013
<i>Sceloporus samcolemanni</i>	Smith and Hall, 1974	LC	High (15)	Wilson et al. 2013
<i>Sceloporus scalaris</i>	Wiegmann, 1828	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus schmidti</i>	Jones, 1927	NE	Medium (11)	McCranie 2018
<i>Sceloporus serrifer</i>	Cope, 1866	NE	Low (6)	Wilson et al. 2013
<i>Sceloporus shannonorum</i>	Langebartel, 1959	NE	High (15)	Wilson et al. 2013
<i>Sceloporus siniferus</i>	Cope, 1870	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus slevini</i>	Smith, 1937	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus smaragdinus</i>	Bocourt, 1873	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus smithi</i>	Hartweg and Oliver, 1937	LC	High (15)	Wilson et al. 2013
<i>Sceloporus spinosus</i>	Wiegmann, 1828	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus squamosus</i>	Bocourt, 1874	LC	Medium (11)	Wilson et al. 2013
<i>Sceloporus stejnegeri</i>	Smith, 1942	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus subniger</i>	Poglayen and Smith, 1958	NE	High (15)	Johnson et al. 2017
<i>Sceloporus subpictus</i>	Lynch and Smith, 1965	DD	High (16)	Wilson et al. 2013
<i>Sceloporus sugillatus</i>	Smith, 1942	LC	High (16)	Wilson et al. 2013
<i>Sceloporus taeniocnemis</i>	Cope, 1885	LC	Medium (12)	Wilson et al. 2013
<i>Sceloporus tanneri</i>	Smith and Larsen, 1975	DD	High (16)	Wilson et al. 2013
<i>Sceloporus teapensis</i>	Günther, 1890	LC	Medium (13)	Wilson et al. 2013
<i>Sceloporus torquatus</i>	Wiegmann, 1828	NE	High (16)*	This study
<i>Sceloporus tristichus</i>	Cope, 1875	NE	Medium (11)	This study
<i>Sceloporus undulatus</i>	(Bosc and Daudin, 1801)	LC	Low (9)	This study
<i>Sceloporus unicanthalis</i>	Smith, 1937	NE	High (16)	Johnson et al. 2017
<i>Sceloporus uniformis</i>	Phelan and Brattstrom, 1955	NE	Medium (13)	Wilson et al. 2013
<i>Sceloporus utiformis</i>	Cope, 1864	LC	High (15)	Wilson et al. 2013
<i>Sceloporus vandenburgianus</i>	Cope, 1896	LC	High (14)	Wilson et al. 2013
<i>Sceloporus variabilis</i>	Wiegmann, 1834	LC	Low (9)*	This study
<i>Sceloporus virgatus</i>	Smith, 1938	LC	High (15)	Wilson et al. 2013
<i>Sceloporus woodi</i>	Stejneger, 1918	NT	High (16)	This study
<i>Sceloporus zosteromus</i>	Cope, 1863	LC	Medium (12)	Wilson et al. 2013

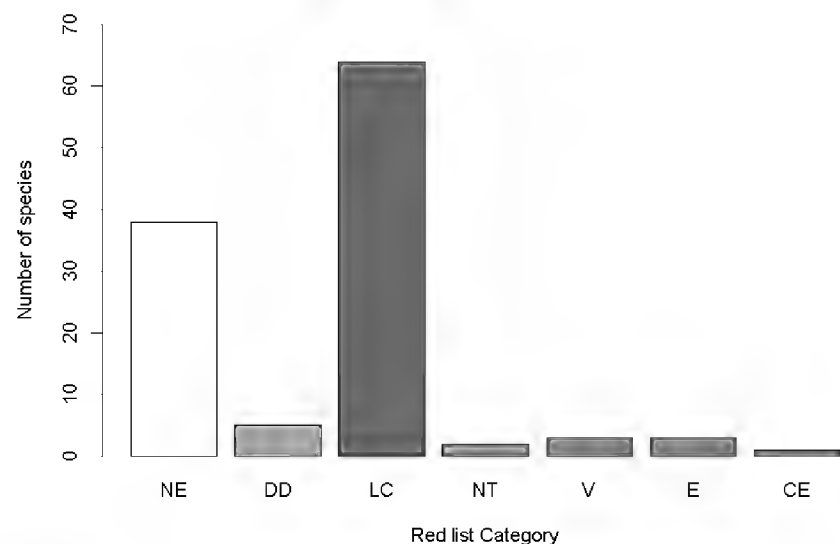


Fig. 1. Number of *Sceloporus* species assigned to each IUCN Red List category: Not Evaluated (NE), Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), and Critically Endangered (CE).

three are Vulnerable, three are Endangered, and one is Critically Endangered (Table 1, Fig. 1). In contrast, using the EVS system, ten species of *Sceloporus* are at the low level, 37 at the medium level, and 69 at the high level (Table 1, Fig. 2). Most of the species in the high EVS category were categorized as either Not Evaluated, Data Deficient, or Least Concern in the IUCN Red List (Fig. 3). This discrepancy between the EVS system and the IUCN Red List is consistent with the findings of other studies (e.g., Böhm et al. 2013, Wilson et al. 2013; Meiri and Chapple 2016; Caetano et al. 2022).

Although the IUCN Red List has been the leading authority on global species extinction risk for five decades (Betts et al. 2019), the assessments of reptiles in general (Böhm et al. 2013; Wilson et al. 2013; Caetano et al. 2022) and lizards in particular (Meiri and Chapple 2016) have lagged behind other groups. Meiri and Chapple (2016) evaluated the biases of the lizards assessed by the IUCN and found that most lizard species (64%) had not been assessed by the IUCN Red List at that time (see also Tingley et al. 2016). In this study, we found that 38 species of *Sceloporus* had not been evaluated for the Red List, or 32% of species in the genus *Sceloporus*. The higher level of assessment of *Sceloporus* could be related

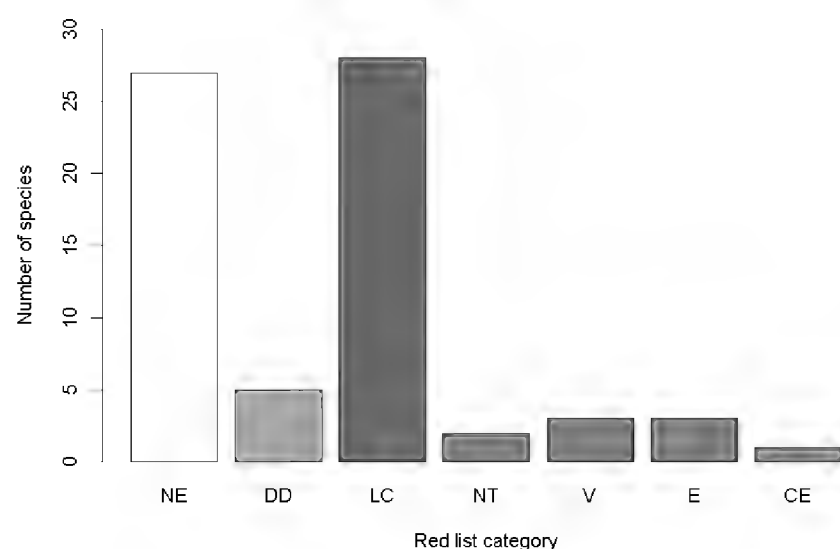


Fig. 3. Number of *Sceloporus* species with high vulnerability in its corresponding IUCN Red List category.

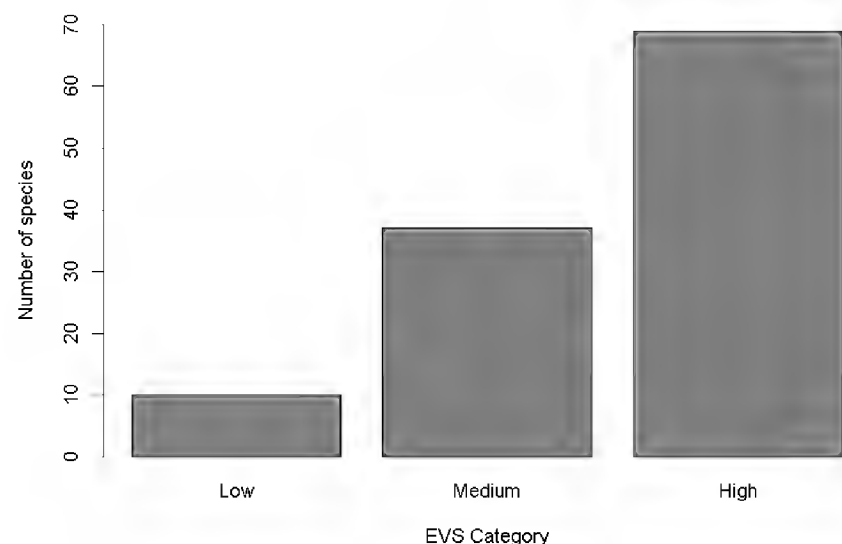


Fig. 2. Number of *Sceloporus* species assigned to each Environmental Vulnerability Score (EVS) category.

to the fact that *Sceloporus* is one of the most widely studied genera of lizards (Sites et al. 1992). Wilson et al. (2013) developed their EVS system specifically to overcome the “assessment gap” (Meiri and Chapple 2016) in reptiles. According to the EVS system, most of the *Sceloporus* species (59%) have high vulnerability, which is consistent with the evaluations made by Wilson et al. (2013), Johnson et al. (2017), and Mata-Silva et al. (2019) for the Mesoamerican herpetofauna.

Conservation Priority and Endemism of *Sceloporus* Lizards

Conservation priority levels were proposed by Johnson et al. (2017) and Mata-Silva et al. (2019) to recognize the species in need of conservation actions due to their high vulnerability and restricted ecological distributions. Our assessment found that 46 species of *Sceloporus* (39%) were assigned to conservation priority level I, the highest priority category (Table 2, Fig. 4). In addition, 84 of the 116 *Sceloporus* species are country endemics (72%). Of these endemic species, 77% are in the first three levels of conservation priority (i.e., 45 in level 1, 13 in level 2, and seven in level 3). Mexico is the country with most endemic species (73 of 84; 86.9%), followed by the USA with seven endemic species (seven of 84; 8.3%), Honduras with three (three of 84; 3.5%), and Guatemala with one (one of 84; 1.2%). These results are consistent with those reported by Johnson et al. (2017), since Mexico is the country with most endemic species of reptiles in North America.

Conclusions

Reptiles have been historically neglected by conservation assessments, and *Sceloporus* lizards are no exception. Even though *Sceloporus* is a widely studied genus of reptiles, a gap in conservation biology studies remains, and this is reflected in the underestimation of conservation

Conservation status of *Sceloporus* lizards

Table 2. Priority conservation level and endemism of *Sceloporus* species. The country abbreviations are: MX = Mexico, USA = United States of America, HND = Honduras, GT = Guatemala, and ne= Not endemic.

Priority 1			
	Endemism		Endemism
<i>Sceloporus adleri</i>	MX	<i>Sceloporus huichol</i>	MX
<i>Sceloporus aeneus</i>	MX	<i>Sceloporus hunsakeri</i>	MX
<i>Sceloporus anahuacus</i>	MX	<i>Sceloporus insignis</i>	MX
<i>Sceloporus angustus</i>	MX	<i>Sceloporus lemosespinali</i>	MX
<i>Sceloporus aurantius</i>	MX	<i>Sceloporus lineatulus</i>	MX
<i>Sceloporus aureolus</i>	MX	<i>Sceloporus macdougalli</i>	MX
<i>Sceloporus becki</i>	USA	<i>Sceloporus maculosus</i>	MX
<i>Sceloporus binocularis</i>	MX	<i>Sceloporus madrensis</i>	MX
<i>Sceloporus caeruleus</i>	MX	<i>Sceloporus mikeprestoni</i>	MX
<i>Sceloporus chaneyi</i>	MX	<i>Sceloporus omiltemanus</i>	MX
<i>Sceloporus cozumelae</i>	MX	<i>Sceloporus ornatus</i>	MX
<i>Sceloporus cryptus</i>	MX	<i>Sceloporus palaciosi</i>	MX
<i>Sceloporus cupreus</i>	MX	<i>Sceloporus prezygus</i>	ne
<i>Sceloporus cyanostictus</i>	MX	<i>Sceloporus samcolemanni</i>	MX
<i>Sceloporus dixonii</i>	MX	<i>Sceloporus schmidtii</i>	HND
<i>Sceloporus druckercolini</i>	MX	<i>Sceloporus shannonorum</i>	MX
<i>Sceloporus esperanzae</i>	HND	<i>Sceloporus subniger</i>	MX
<i>Sceloporus exsul</i>	MX	<i>Sceloporus subpictus</i>	MX
<i>Sceloporus gadsdeni</i>	MX	<i>Sceloporus sugillatus</i>	MX
<i>Sceloporus geminus</i>	MX	<i>Sceloporus tanneri</i>	MX
<i>Sceloporus goldmani</i>	MX	<i>Sceloporus torquatus</i>	MX
<i>Sceloporus grandaevus</i>	MX	<i>Sceloporus unicanthalis</i>	MX
<i>Sceloporus halli</i>	MX		
<i>Sceloporus hesperus</i>	MX		
Priority 2		Priority 3	
	Endemism		Endemism
<i>Sceloporus albiventris</i>	MX	<i>Sceloporus formosus</i>	MX
<i>Sceloporus arenicolus</i>	USA	<i>Sceloporus malachiticus</i>	ne
<i>Sceloporus asper</i>	MX	<i>Sceloporus megalepidurus</i>	MX
<i>Sceloporus brownorum</i>	MX	<i>Sceloporus melanogaster</i>	MX
<i>Sceloporus bulleri</i>	MX	<i>Sceloporus oberon</i>	MX
<i>Sceloporus cautus</i>	MX	<i>Sceloporus parvus</i>	MX
<i>Sceloporus couchii</i>	MX	<i>Sceloporus smithi</i>	MX
<i>Sceloporus cyanogenys</i>	ne	<i>Sceloporus utiformis</i>	MX
<i>Sceloporus edwardtaylori</i>	MX		
<i>Sceloporus heterolepis</i>	MX		
<i>Sceloporus lunae</i>	GT		
<i>Sceloporus lundelli</i>	ne		
<i>Sceloporus minor</i>	MX		
<i>Sceloporus salvini</i>	MX		
<i>Sceloporus vandenburgianus</i>	ne		
<i>Sceloporus virgatus</i>	ne		
<i>Sceloporus woodi</i>	USA		

Table 2 (continued). Priority conservation level and endemism of *Sceloporus* species. The country abbreviations are: MX = Mexico, USA = United States of America, HND = Honduras, GT = Guatemala, and ne= Not endemic.

Priority 7		Priority 8	
	Endemism		Endemism
<i>Sceloporus licki</i>	MX	<i>Sceloporus bicanthalis</i>	MX
<i>Sceloporus zosteromus</i>	MX	<i>Sceloporus dugesii</i>	MX
		<i>Sceloporus hondurensis</i>	HND
		<i>Sceloporus ochoterenae</i>	MX
		<i>Sceloporus stejnegeri</i>	MX
		<i>Sceloporus uniformis</i>	ne
Priority 9		Priority 10	
	Endemism		Endemism
<i>Sceloporus chrysostictus</i>	ne	<i>Sceloporus carinatus</i>	ne
<i>Sceloporus cowlesi</i>	ne	<i>Sceloporus clarkii</i>	ne
<i>Sceloporus gadoviae</i>	MX	<i>Sceloporus horridus</i>	MX
<i>Sceloporus jalapae</i>	MX	<i>Sceloporus nelsoni</i>	MX
<i>Sceloporus jarrovii</i>	ne	<i>Sceloporus poinsettii</i>	ne
<i>Sceloporus marmoratus</i>	ne	<i>Sceloporus smaragdinus</i>	ne
<i>Sceloporus merriami</i>	ne	<i>Sceloporus spinosus</i>	MX
<i>Sceloporus mucronatus</i>	MX	<i>Sceloporus squamosus</i>	ne
<i>Sceloporus occidentalis</i>	ne	<i>Sceloporus taeniocnemis</i>	ne
<i>Sceloporus olivaceus</i>	ne		
<i>Sceloporus pyrocephalus</i>	MX		
<i>Sceloporus scalaris</i>	MX		
<i>Sceloporus siniferus</i>	ne		
<i>Sceloporus slevini</i>	ne		
<i>Sceloporus teapensis</i>	ne		
Priority 11		Priority 12	
	Endemism		Endemism
<i>Sceloporus internasalis</i>	ne	<i>Sceloporus tristichus</i>	USA
Priority 13		Priority 17	
	Endemism		Endemism
<i>Sceloporus acanthinus</i>	ne	<i>Sceloporus grammicus</i>	ne
		<i>Sceloporus magister</i>	ne
		<i>Sceloporus melanorhinus</i>	ne
		<i>Sceloporus olloporus</i>	ne
Priority 18			
	Endemism		
<i>Sceloporus consobrinus</i>	USA		
<i>Sceloporus graciosus</i>	USA		
<i>Sceloporus orcutti</i>	ne		

risk assessments by the IUCN Red List. In this study, we found that 31% of *Sceloporus* species have not been evaluated by IUCN. Also, ~80% of species with high vulnerability based on the EVS (69 species) are either Not Evaluated (38%) or listed as Least Concern (41%) on the IUCN Red List. This underestimation could be related to the cryptic nature of most *Sceloporus* lizards, which have narrow distribution ranges and highly specific ecological requirements, and are usually inconspicuous. In contrast to the IUCN Red List, the EVS system can

easily evaluate less well studied species and considers three important aspects of species conservation risk assessment: distribution range, ecological versatility, and anthropogenic pressures. Implementing conservation risk assessments such as the EVS system is imperative for rapid evaluations and timely conservation actions for *Sceloporus* lizards. The results of this survey also emphasize that greater efforts need to be expended to fully understand the true conservation status of species in the genus *Sceloporus*, as well as the specific threats

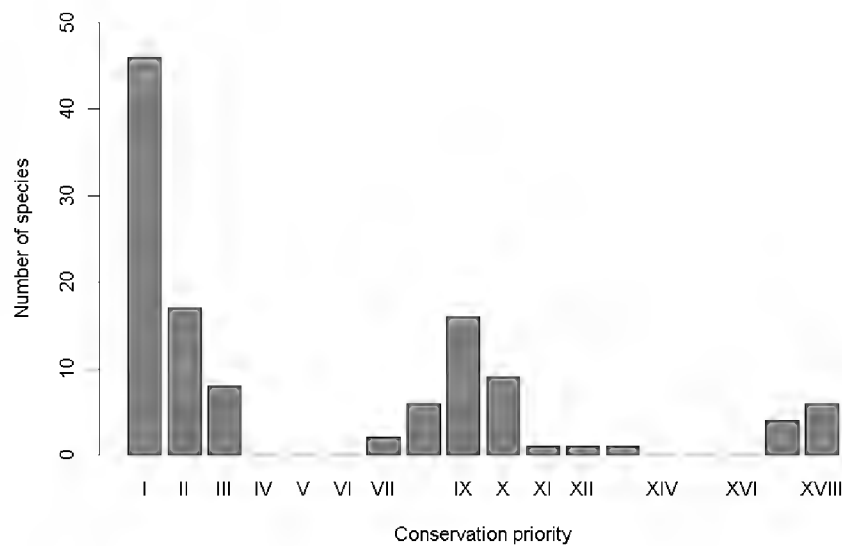


Fig. 4. Number of *Sceloporus* species assigned to each conservation priority level. Red bars indicate priority levels with high Environmental Vulnerability Score (EVS) species, orange bars represent priority levels with medium EVS species, and yellow bars indicate priority levels with low EVS species.

that they face.

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Literature Cited

- Barve N, Barve V, Jiménez-Valverde A, Lira-Noriega A, Maher SP, Peterson AT, Soberón J, Villalobos F. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* 222: 1,810–1,819.
- Betts J, Young RP, Hilton-Taylor C, Hoffmann M, Rodríguez JP, Stuart SN, Milner-Gulland EJ. 2019. A framework for evaluating the impact of the IUCN Red List of Threatened Species. *Conservation Biology* 34: 632–643.
- Böhm M, Collen B, Baillie JEM, Bowles P, Chanson J, Cox N, Hammerson G, Hoffmann M, Livingstone SR, Ram M, et al. 2013. The conservation status of the world's reptiles. *Biological Conservation* 157: 372–385.
- Böhm M, Williams R, Bramhall HR, McMillan KM, Davidson AD, Garcia A, Bland LM, Bielby J, Collen B. 2016. Extinction risk correlates in squamate reptiles. *Global Ecology and Biogeography* 25: 391–405.
- Bryson RW, Grummer JA, Connors EM, Tirpak J, McCormack JE, Klicka J. 2021. Cryptic diversity across the Trans-Mexican Volcanic Belt of Mexico in the Montane Bunchgrass Lizard *Sceloporus subniger* (Squamata: Phrynosomatidae). *Zootaxa* 4963(2): 335–353.
- Caetano GHdO, Chapple DG, Grenyer R, Raz T, Rosenblatt J, Tingley R, Böhm M, Meiri S, Roll U. 2022. Automated assessment reveals that the extinction risk of reptiles is widely underestimated across space and phylogeny. *PLoS Biology* 20(5): e3001544.
- Campillo-García G, Flores-Villela O, Butler BO, Velasco Vinasco JA, Ramírez Corona F. 2021. Hidden diversity within a polytypic species: the enigmatic *Sceloporus torquatus* Wiegmann, 1828 (Reptilia, Squamata, Phrynosomatidae). *Vertebrate Zoology* 71: 781–798.
- Campillo-García G, Flores-Villela O, Butler BO, Benabib M, Castiglia R. 2023. More cryptic diversity among spiny lizards of the *Sceloporus torquatus* complex discovered through a multilocus approach. *Amphibia-Reptilia* 45: 21–35.
- Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM. 2015. Accelerated modern human-induced species losses: entering the sixth mass extinction. *Science Advances* 1: e1400253.
- Chan LM, Archie JW, Yoder AD, Fitzgerald LA. 2013. Review of the systematic status of *Sceloporus arenicolus* Degenhardt and Jones, 1972, with an estimate of divergence time. *Zootaxa* 3664(3): 312–320.
- Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. 2014. Defaunation in the Anthropocene. *Science* 345(6195): 401–406.
- Ernst JA, Branch LC, Clark AM, Hokit DG. 2004. Polymorphic microsatellite markers for the Florida Scrub Lizard (*Sceloporus woodi*). *Molecular Ecology Notes* 4: 364–365.
- Fitzgerald LA, Walkup D, Chyn K, Buchholtz E, Angeli N, Parker M. 2018. The future for reptiles: advances and challenges in the Anthropocene. Pp. 163–174 In: *Encyclopedia of the Anthropocene*. Editors, DellaSala D, Goldstein M. Elsevier Science, Oxford, United Kingdom. 2,280 p.
- Flores-Villela O, Smith EN, Campillo-García G, Martínez-Méndez N, Campbell JA. 2022. A new species of *Sceloporus* of the torquatus group (Reptilia: Phrynosomatidae) from West Mexico. *Zootaxa* 5134(2): 286–296.
- Gadsden H, Castañeda G, Huitrón-Ramírez RA, Zapata-Aguilera SA, Ruíz S, Smith GR. 2018. Ecology of *Sceloporus gadsdeni* (Squamata: Phrynosomatidae) from the central Chihuahuan Desert, Mexico. *Phyllomedusa: Journal of Herpetology* 17(2): 181–193.
- García-Padilla E, DeSantis DL, Rocha A, Mata-Silva V, Johnson JD, Wilson LD. 2020. Conserving the Mesoamerican herpetofauna: the most critical case of the priority level one endemic species. *Amphibian & Reptile Conservation* 14(2) [General Section]: 73–132 (e240).
- Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, et al. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience* 50(8): 653–666.

- Guisan A, Zimmermann NE. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* 135: 147–186.
- Hall WP. 2009. Chromosome variation, genomics, speciation, and evolution in *Sceloporus* lizards. *Cytogenetic and Genome Research* 127(2–4): 143–165.
- Hokit DG, Branch LC. 2003. Habitat patch size affects demographics of the Florida Scrub Lizard (*Sceloporus woodi*). *Journal of Herpetology* 37(2): 257–265.
- IPBES. 2019. *Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*. Editors, Brondizio ES, Settele J, Díaz S, Ngo HT. IPBES Secretariat, Bonn, Germany. 1,148 p.
- Johnson JD, Mata-Silva V, Wilson LD. 2015. A conservation reassessment of the Central American herpetofauna based on the EVS measure. *Amphibian & Reptile Conservation* 9(2) [General Section]: 1–94 (e100).
- Johnson JD, Wilson LD, Mata-Silva V, García-Padilla E, DeSantis DL. 2017. The endemic herpetofauna of Mexico: organisms of global significance in severe peril. *Mesoamerican Herpetology* 4: 544–620.
- Kass JM, Pinilla-Buitrag GE, Paz A, Johnson BA, Grisales-Betancur V, Meenan SI, Attali D, Broennimann O, Galante PJ, Maitner BS, et al. 2023. Wallace 2: a shiny app for modeling species niches and distributions redesigned to facilitate expansion via module contributions. *Ecography* 2023: e06547.
- Lance SL, Hagen C, Glenn TC, Freidenfelds NA, Langkilde T. 2009. Development and characterization of 17 polymorphic microsatellite loci in the eastern fence lizard, *Sceloporus undulatus*. *Conservation Genetics Resources* 1(1): 233–236.
- Lawing AM, Polly PD, Hews DK, Martins EP. 2016. Including fossils in phylogenetic climate reconstructions: a deep time perspective on the climatic niche evolution and diversification of spiny lizards (*Sceloporus*). *American Naturalist* 188: 133–148.
- Leaché AD, Banbury BL, Linkem CW, Nieto-Montes de Oca A. 2016. Phylogenomics of a rapid radiation: Is chromosomal evolution linked to increased diversification in North American spiny lizards (genus *Sceloporus*)? *BMC Evolutionary Biology* 16: 63.
- Martínez-Méndez N, Mejía O, Rocha-Gómez A, Méndez-De La Cruz FR. 2012. Morphological convergence and molecular divergence: the taxonomic status of *Sceloporus serrifer* (Squamata, Phrynosomatidae) subspecies. *Zoologica Scripta* 41: 97–108.
- Mata-Silva V, DeSantis DL, García-Padilla E, Johnson JD, Wilson LD. 2019. The endemic herpetofauna of Central America: a casualty of anthropocentrism. *Amphibian & Reptile Conservation* 13(1) [General Section]: 1–64 (e168).
- Mayani-Parás F, Botello F, Castañeda S, Munguía-Carrara M, Sánchez-Cordero V. 2022. Extinction risk assessment of the endemic terrestrial vertebrates in Mexico. *Biological Conservation* 270: 109562.
- Meiri S, Chapple DG. 2016. Biases in the current knowledge of threat status in lizards, and bridging the ‘assessment gap.’ *Biological Conservation* 204: 6–15.
- Phillips S, Anderson RH, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.
- R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rurik AJ, Wilmoth SC, Dayton KE, Sparkman AM. 2022. Rock refuges are strongly associated with increased urban occupancy in the Western Fence Lizard, *Sceloporus occidentalis*. *Diversity* 14(8): 655.
- Saha A, McRae L, Dodd Jr CK, Gadsden H, Hare KM, Lukoschek V, Böhm M. 2018. Tracking population trends: population time-series data and living planet index for reptiles. *Journal of Herpetology*. 52(3): 259–268.
- Sinervo B, Mendez de la Cruz F, Miles DDB, Heulin B, Sites Jr JW, et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328(5980): 894–899.
- Sites Jr. JW, Archie JW, Cole CJ, Vilella OF. 1992. A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): implications for ecological and evolutionary studies. *Bulletin of the American Museum of Natural History* 213: 1–110.
- Solis-Zurita C, De Luna E, González D. 2019. Phylogenetic relationships in the *Sceloporus variabilis* (Squamata: Phrynosomatidae) complex based on three molecular markers, continuous characters, and geometric morphometric data. *Zoologica Scripta* 48: 419–439.
- Thawley CJ, Langkilde T. 2016. Invasive Fire Ant (*Solenopsis invicta*) predation of Eastern Fence Lizard (*Sceloporus undulatus*) eggs. *Journal of Herpetology* 50(2): 284–288.
- Tingley R, Meiri S, Chapple DG. 2016. Addressing knowledge gaps in reptile conservation. *Biological Conservation* 204: 1–5.
- Todd BD, Willson JD, Gibbons JW. 2010. The global status of reptiles and causes of their decline. Pp. 47–67 In: *Ecotoxicology of Amphibians and Reptiles*. Editors, Sparling DW, Bishop CA, Krest S. CRC Press, Boca Raton, Florida, USA. 944 p.
- Trumbo DR, Funk WC, Pauly GB, Robertson JM. 2021. Conservation genetics of an island-endemic lizard: low Ne and the critical role of intermediate temperatures for genetic connectivity. *Conservation Genetics* 22: 783–797.
- Uetz P, Freed P, Aguilar R, Reyes F, Hošek J. 2022. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 30 January 2022].

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VanDerWal J, Shoo LP, Graham C, Williams E. 2009. Selecting pseudo-absence data for presence-only distribution modeling: How far should you stray from what you know? *Ecological Modelling* 220: 589–594.

Walkup DK, Ryberg WA, Fitzgerald LA, Hibbitts TJ. 2018. Occupancy and detection of an endemic habitat specialist, the Dunes Sagebrush Lizard (*Sceloporus arenicolus*). *Herpetological Conservation and Biology* 13(3): 497–506.

Wiken E, Jiménez Nava F, Griffith G. 2011. *North*

American Terrestrial Ecoregions-Level III. Commission for Environmental Cooperation, Montreal, Canada. 149 p.

Wilson LD, Mata-Silva V, Johnson JD. 2013. A conservation reassessment of the reptiles of Mexico based on the EVS measure. *Amphibian & Reptile Conservation* 7(1): 1–47 (e61).

Wilson LD, McCranie JR. 2004. The conservation status of the herpetofauna of Honduras. *Amphibian & Reptile Conservation* 3(1): 6–33 (e12).



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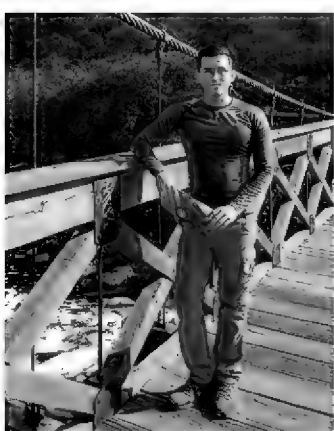
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Conservation status of *Sceloporus* lizards

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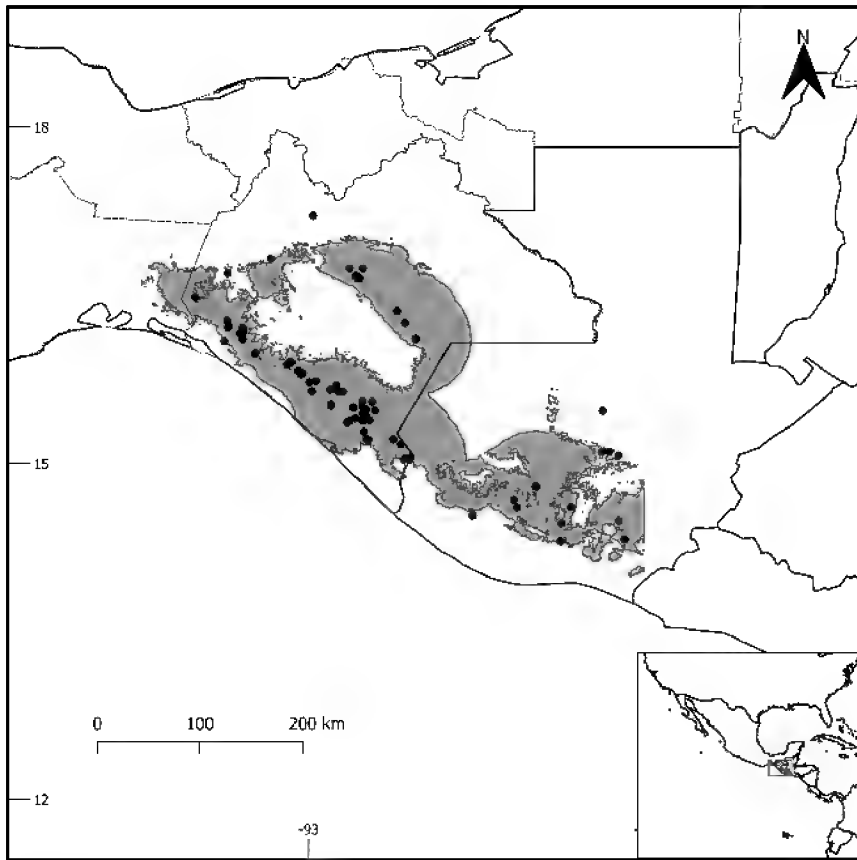
^g Red de Diversidad Biológica del Occidente Mexicano, Instituto de Ecología A. C., Centro Regional del Bajío. Prolongación Lázaro Cárdenas 253, Centro, 61600, Pátzcuaro, Michoacán, México.

e-mail: hgadsden@gmail.com

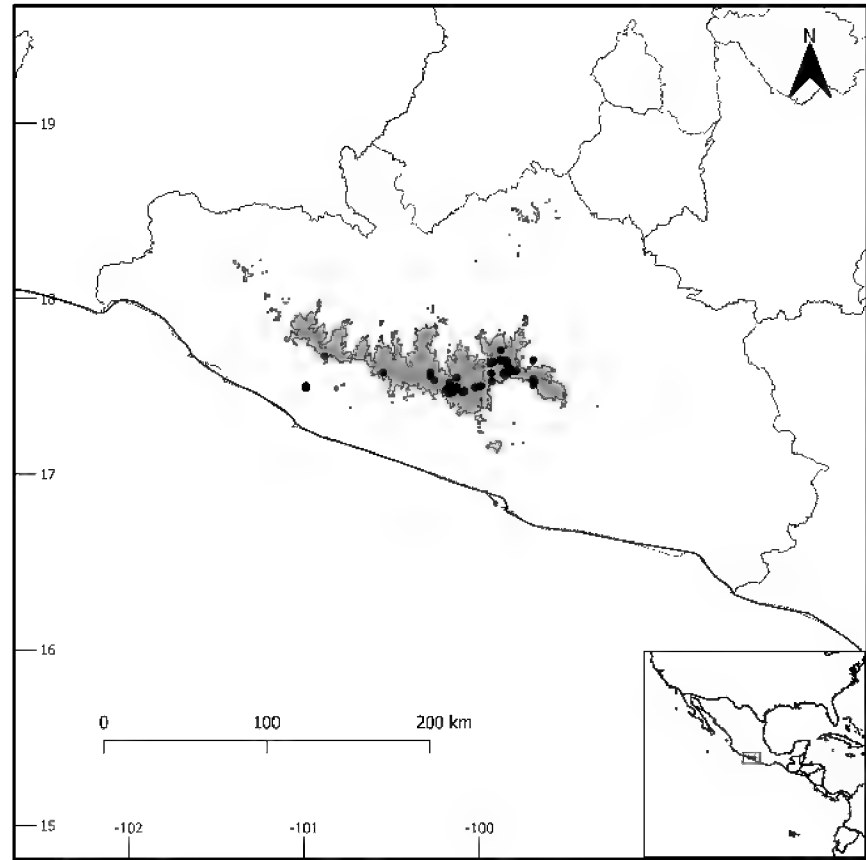
^h Lindale St. s/n, Abbotsford, British Columbia, Canada

Supplementary material

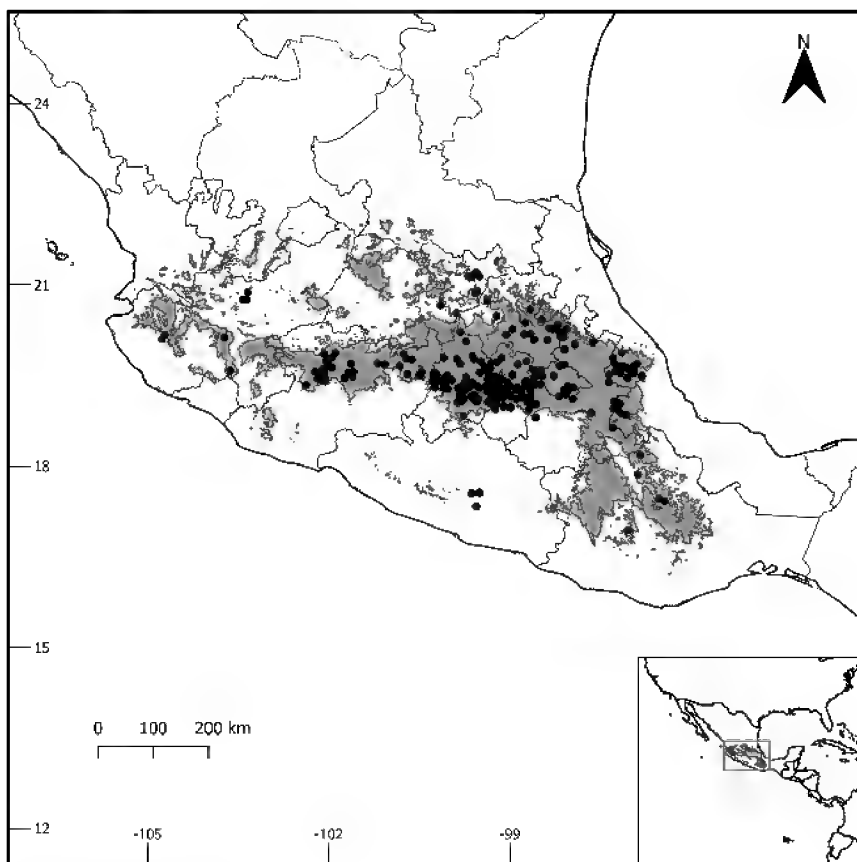
Distribution maps of *Sceloporus* species obtained by ecological niche modelling. In species with less than 5 occurrences, only points of occurrence are shown.



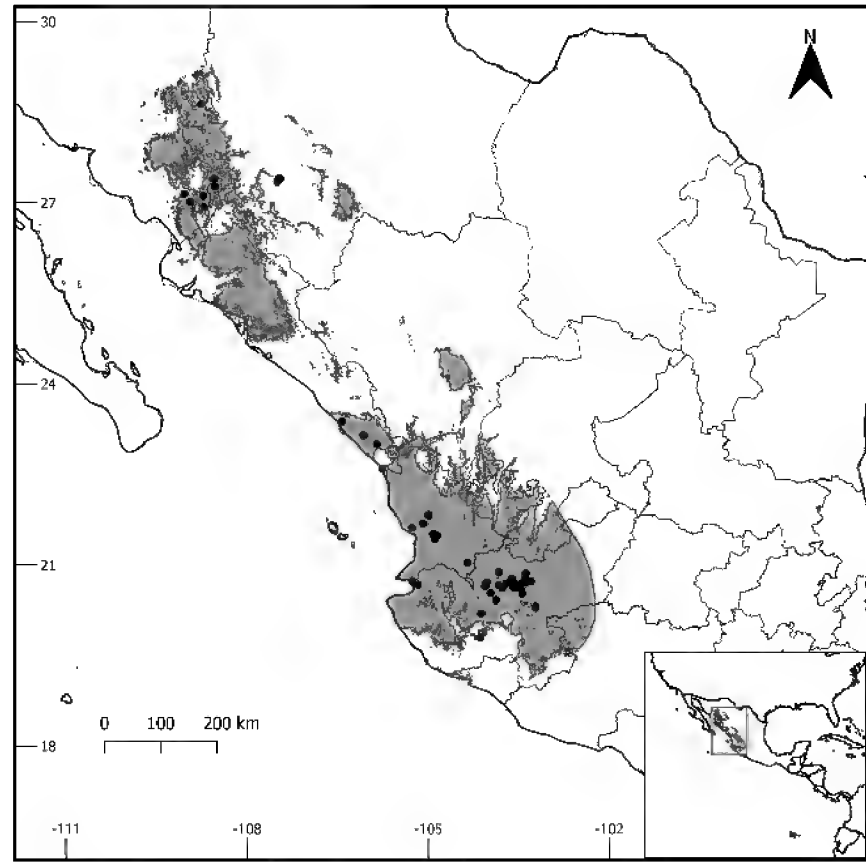
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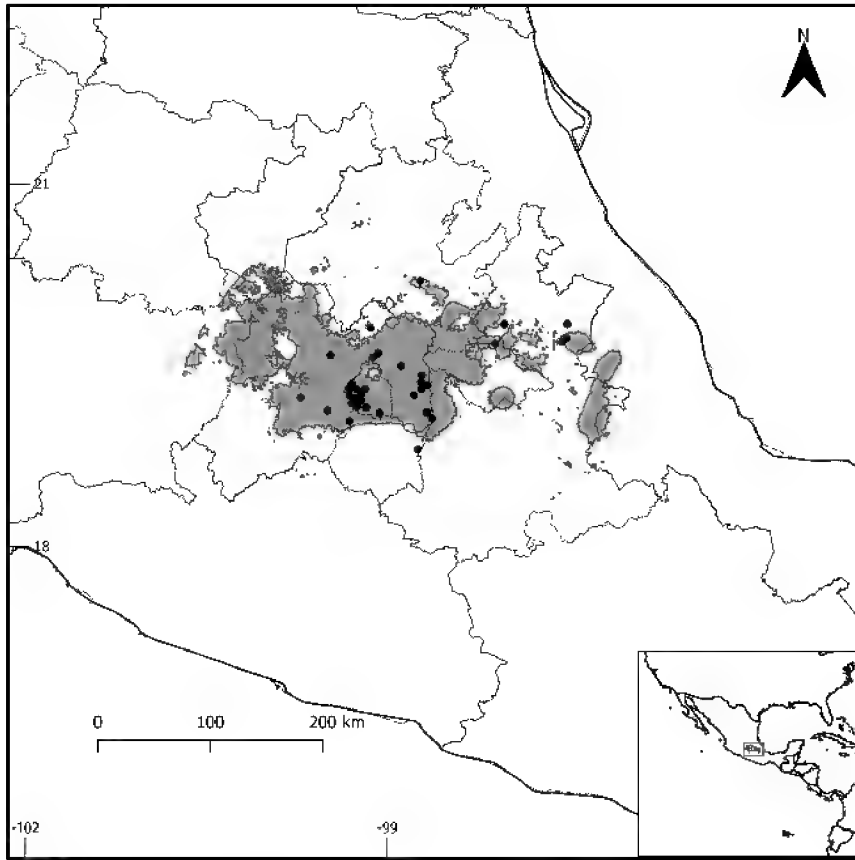
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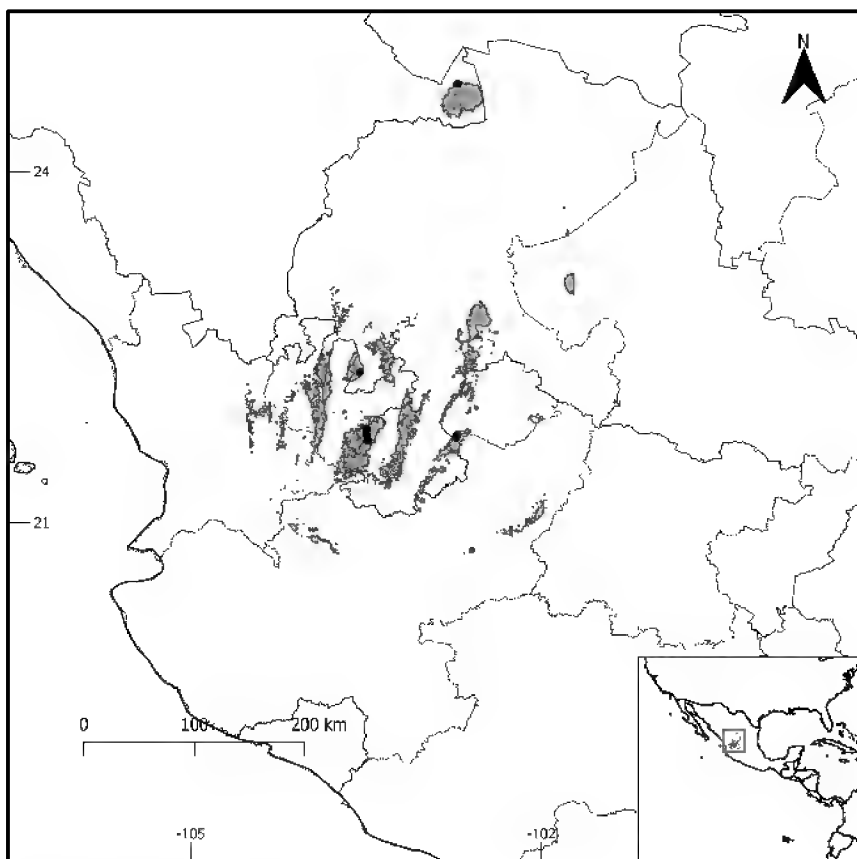


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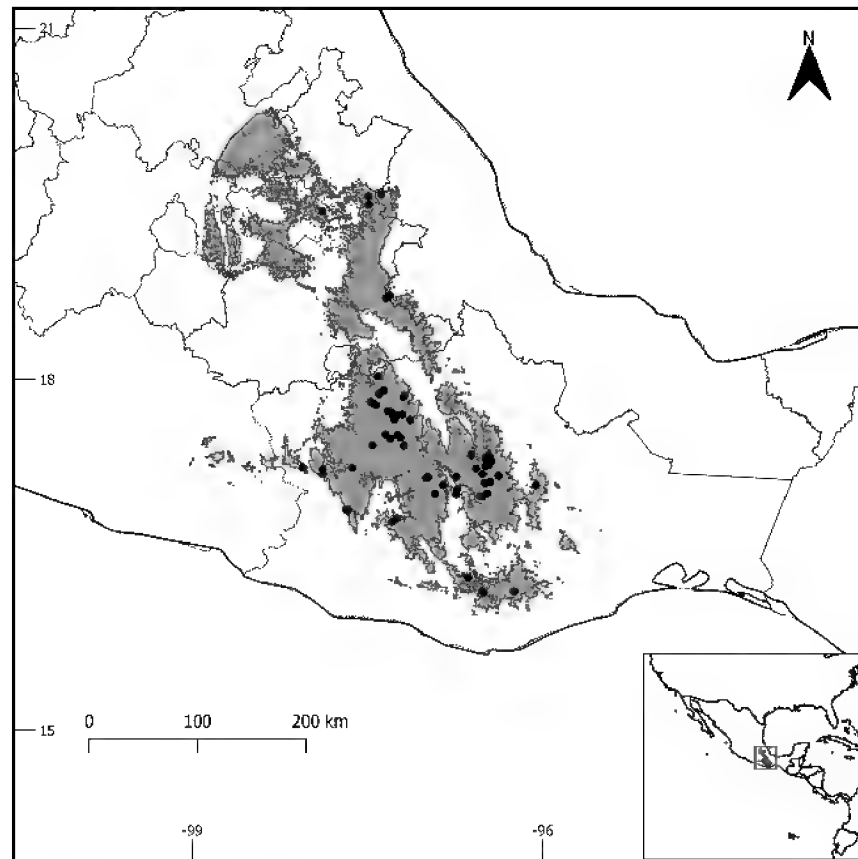


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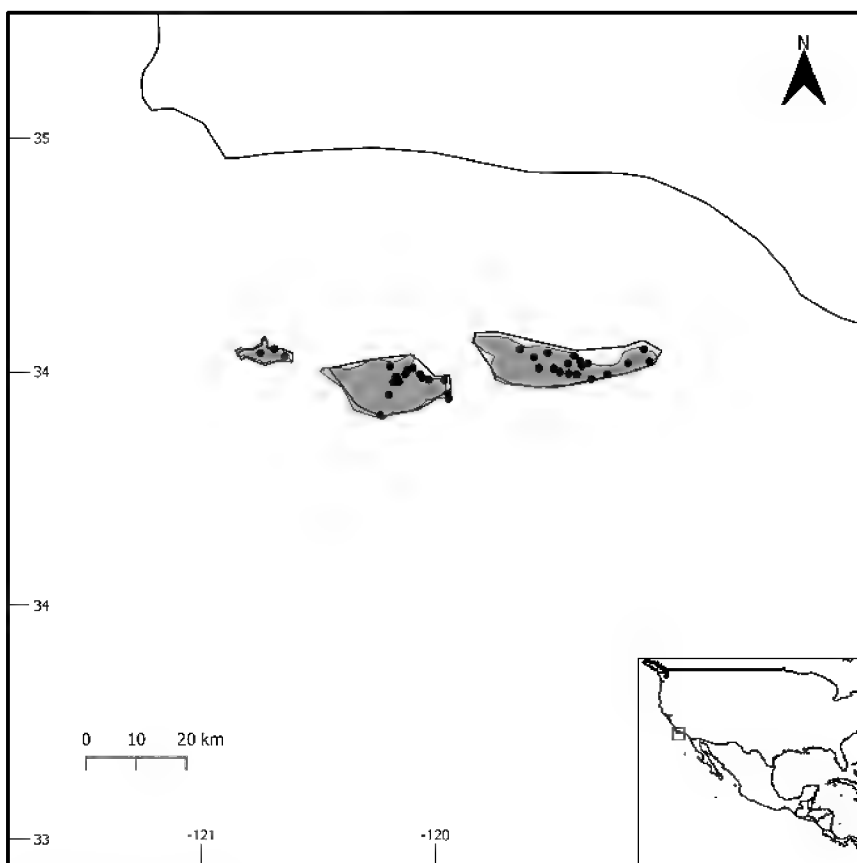




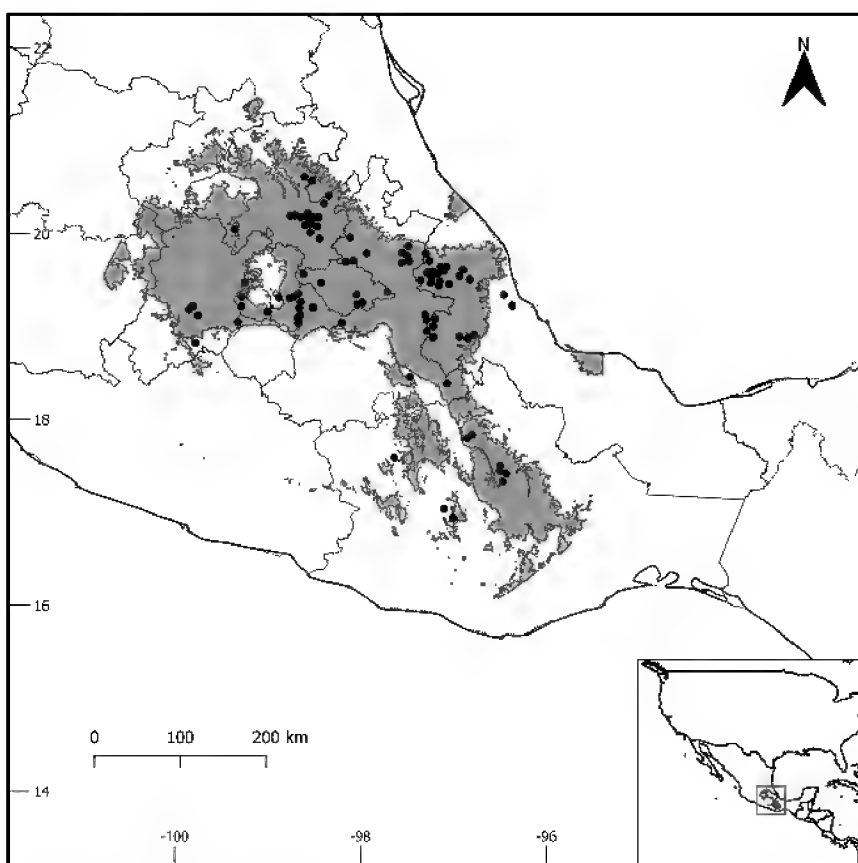
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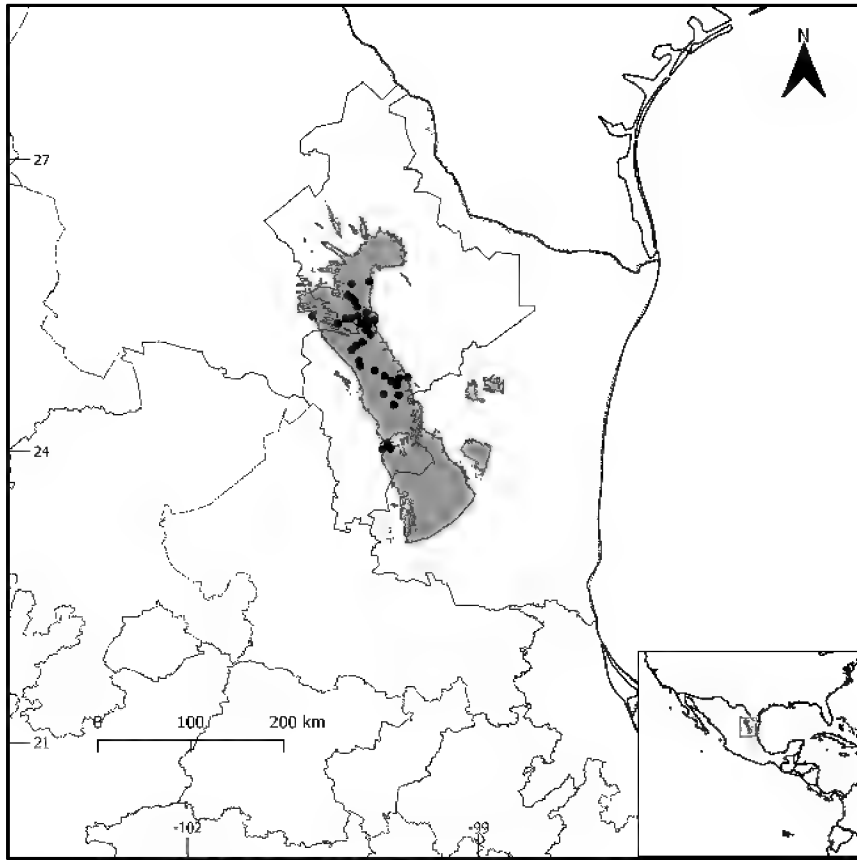
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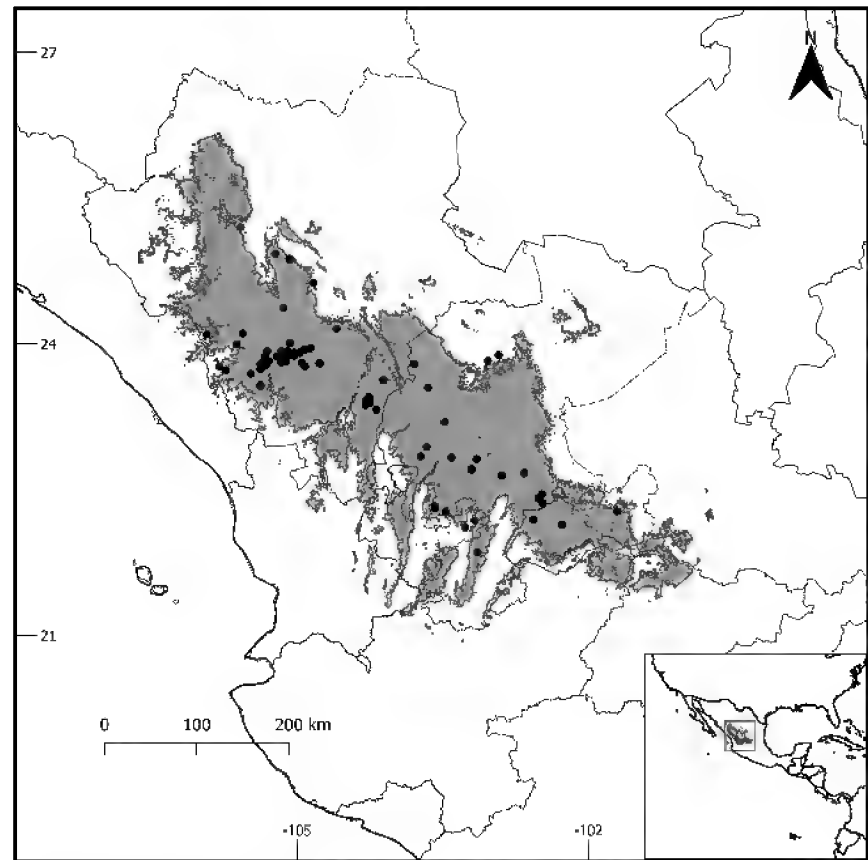
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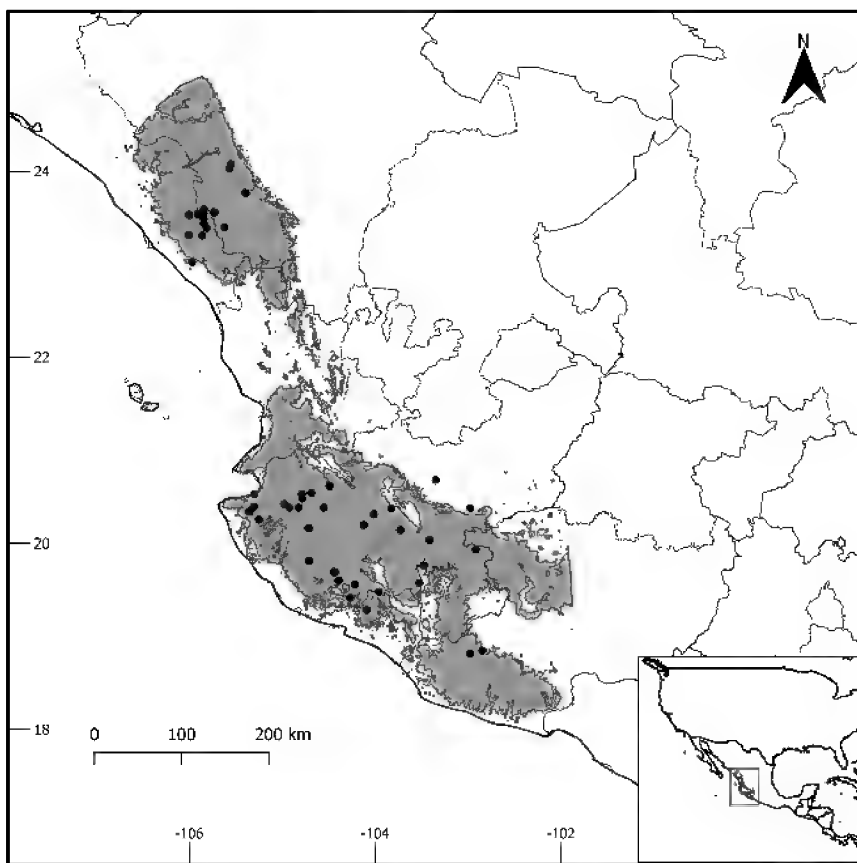
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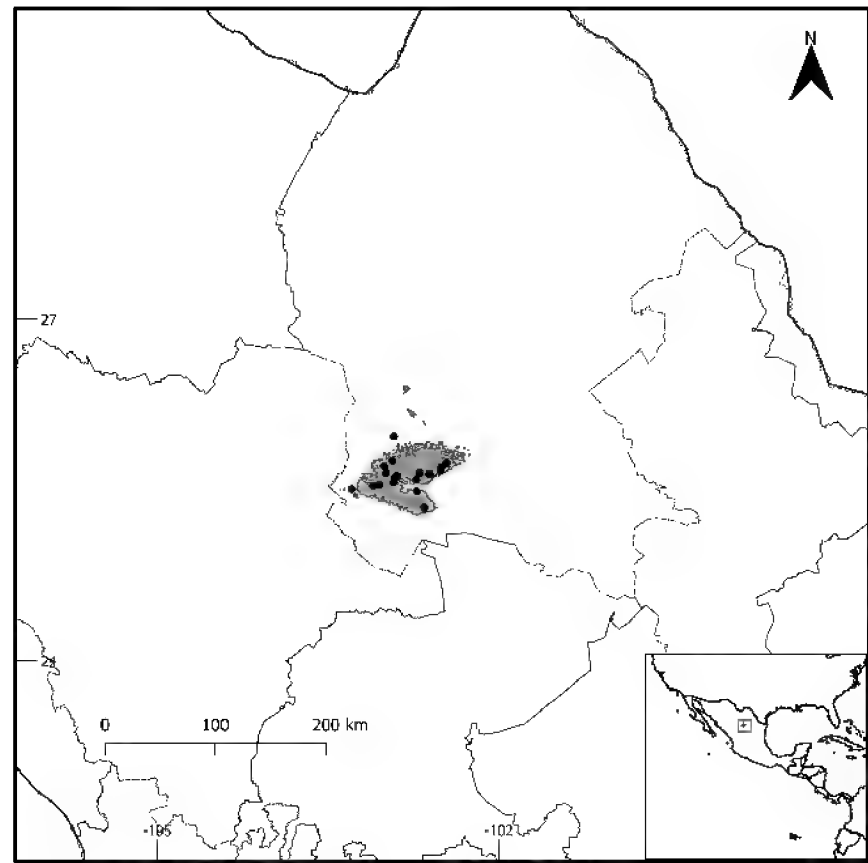
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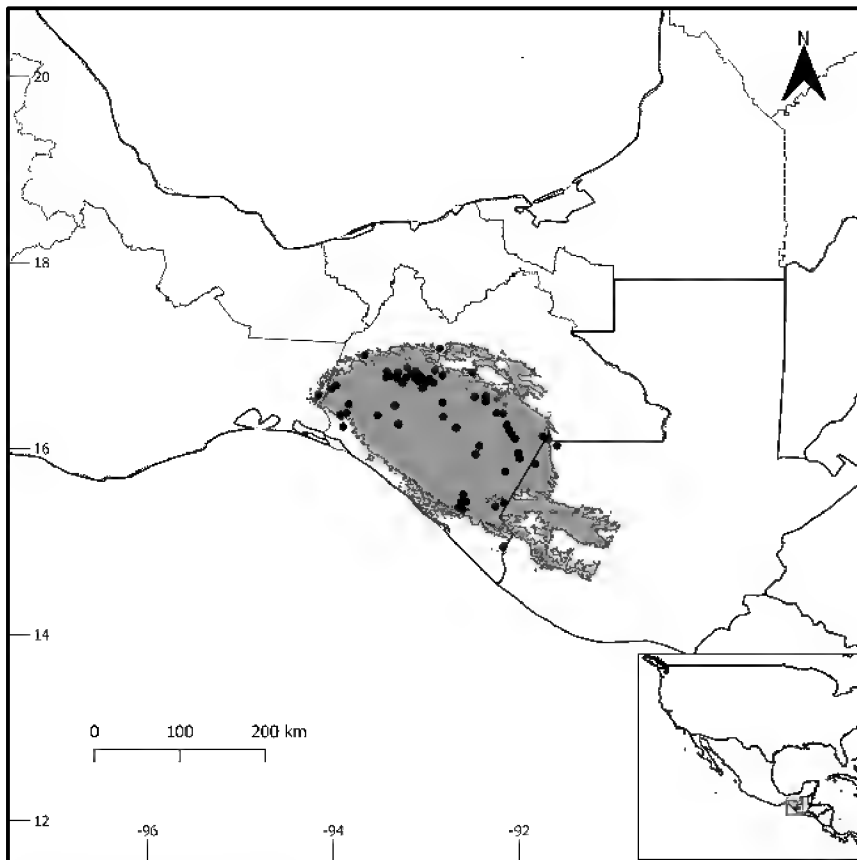
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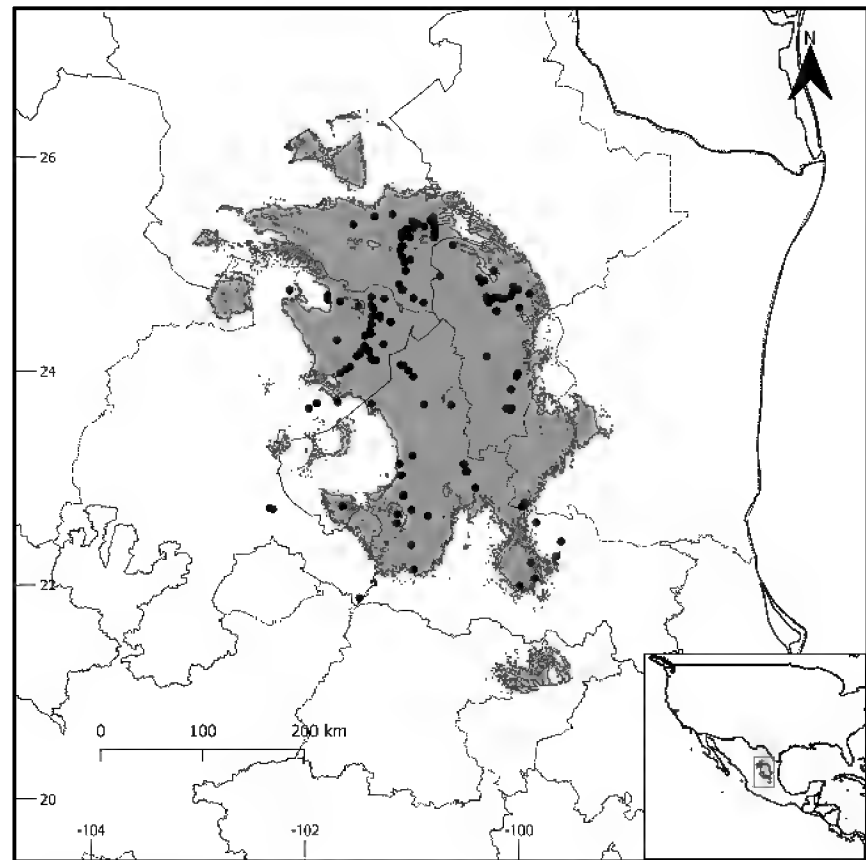
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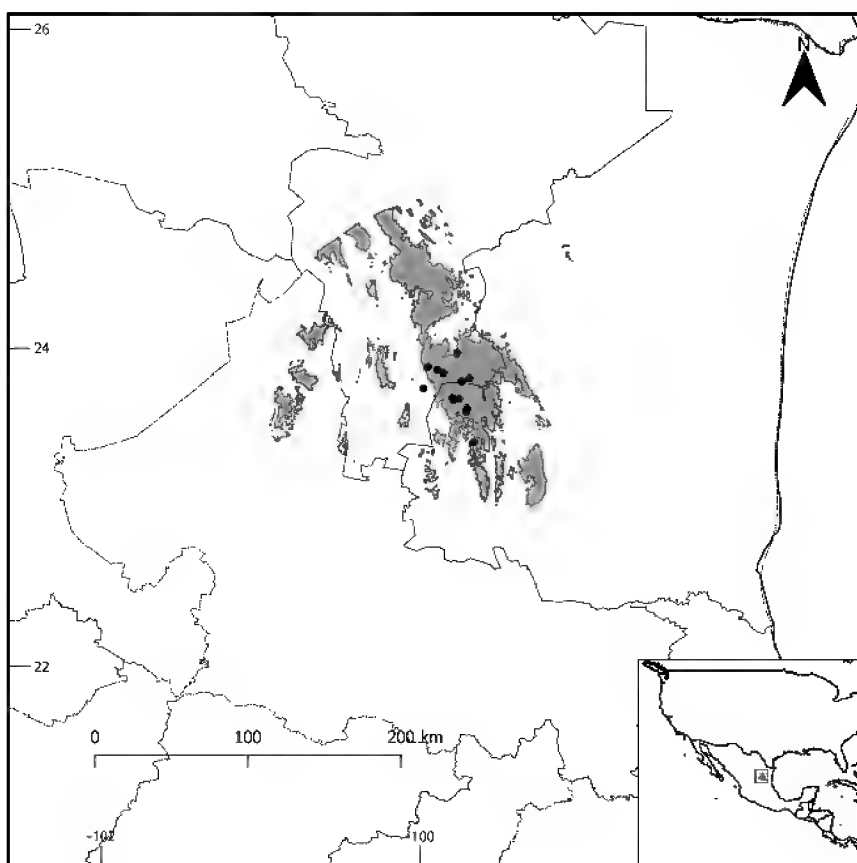
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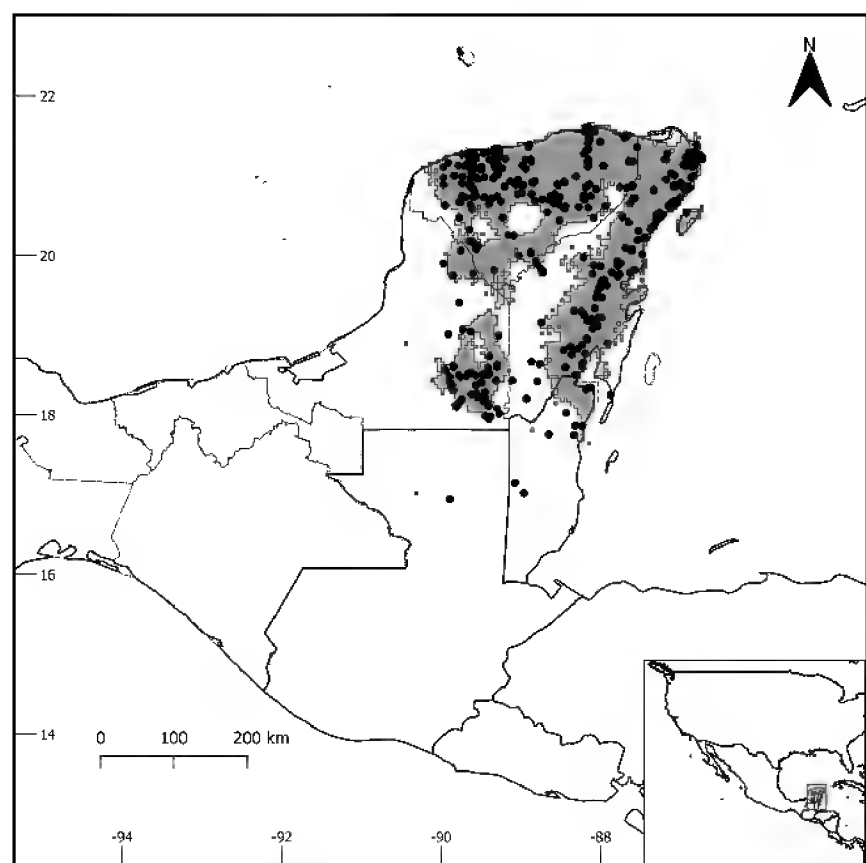
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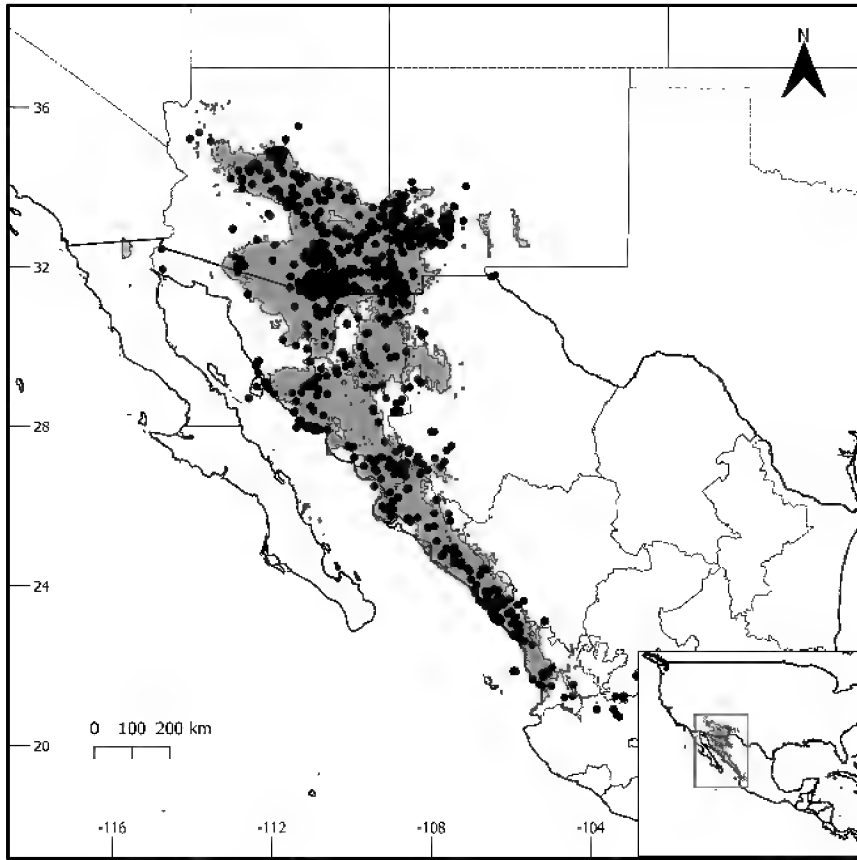
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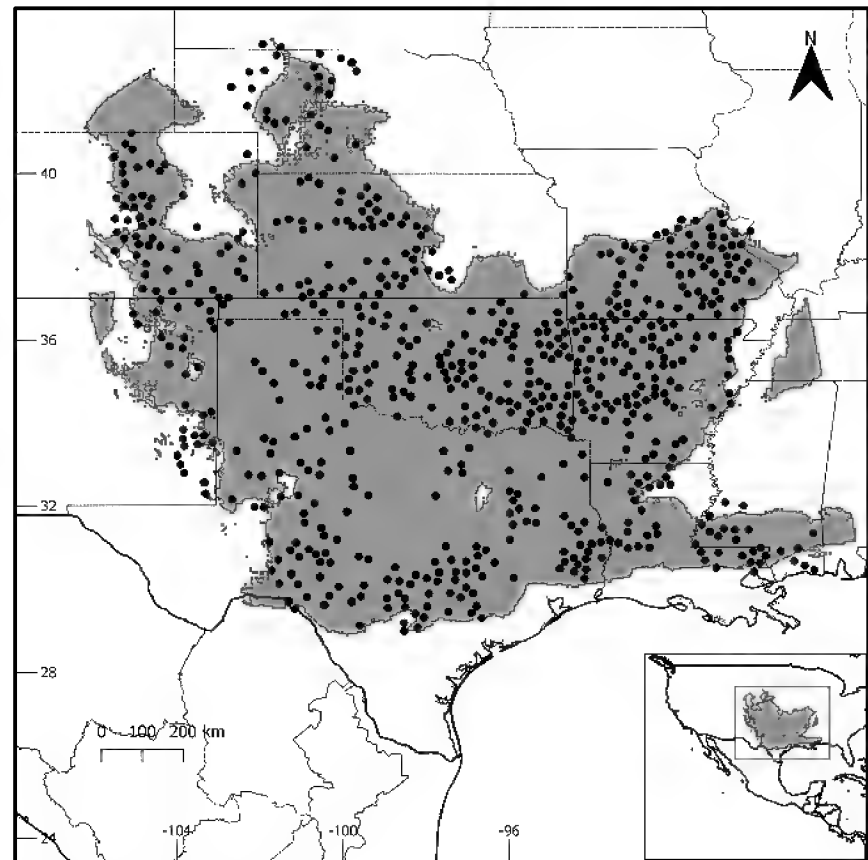
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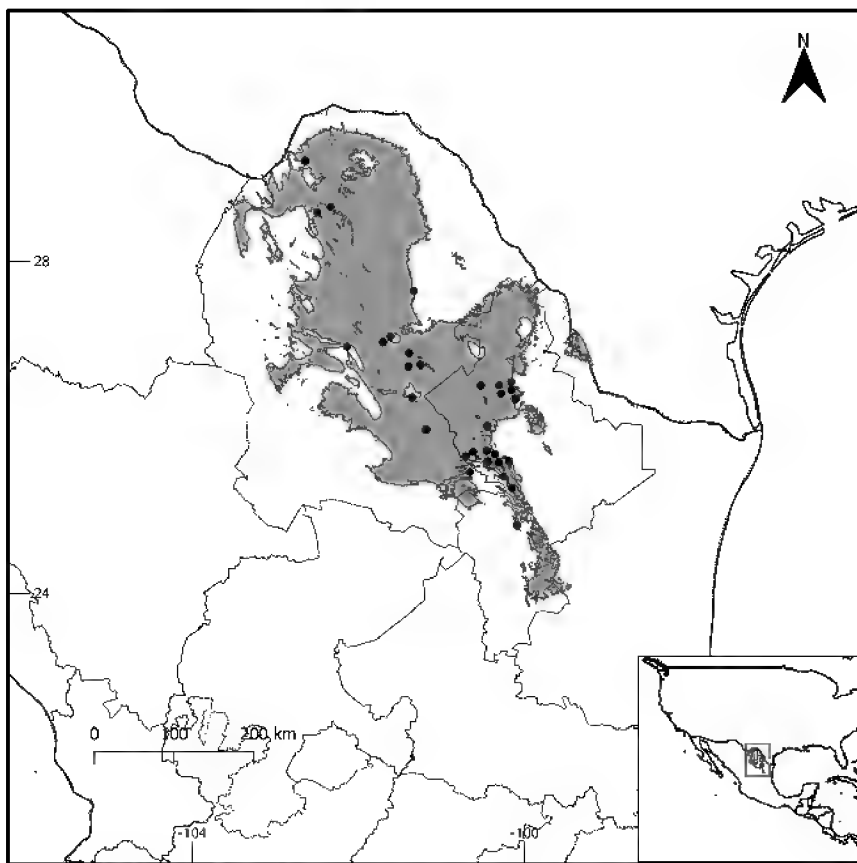
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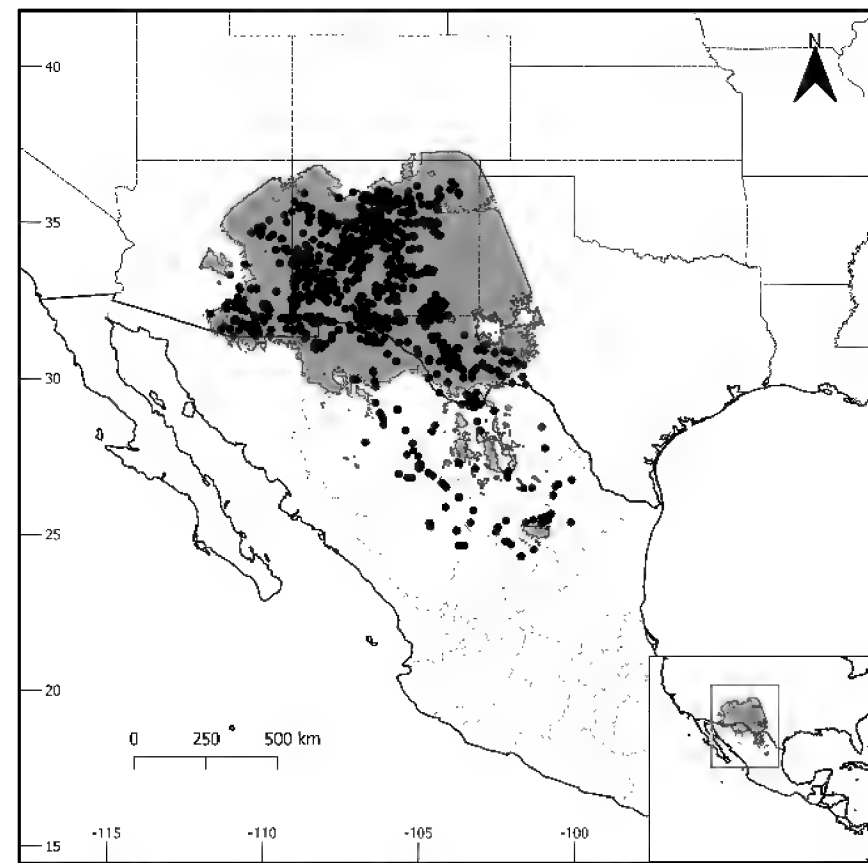
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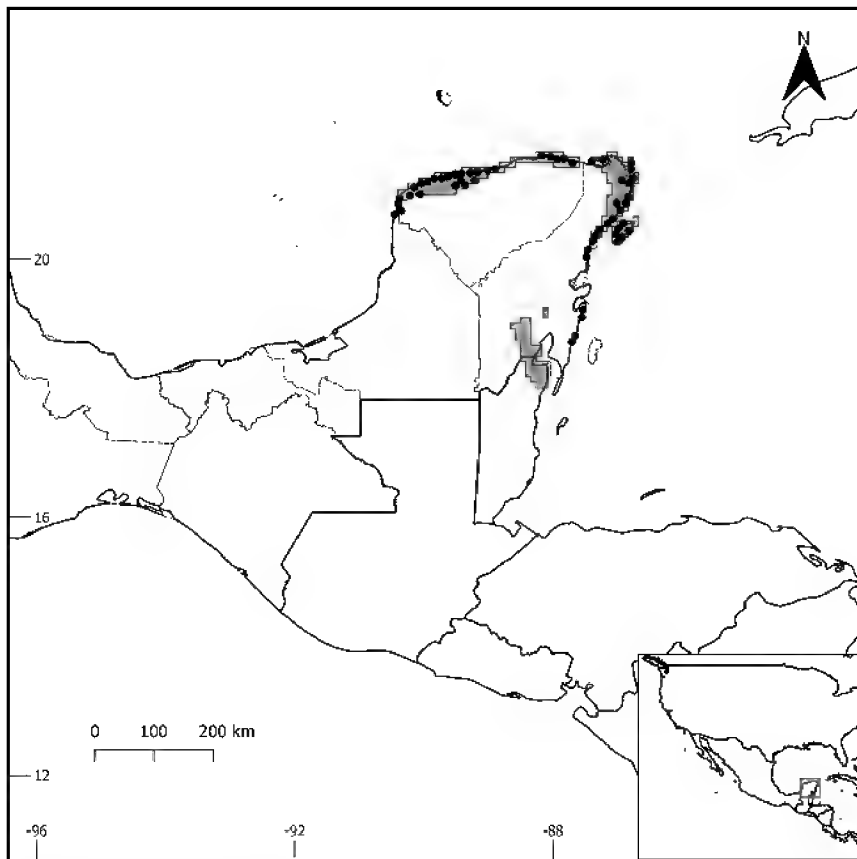
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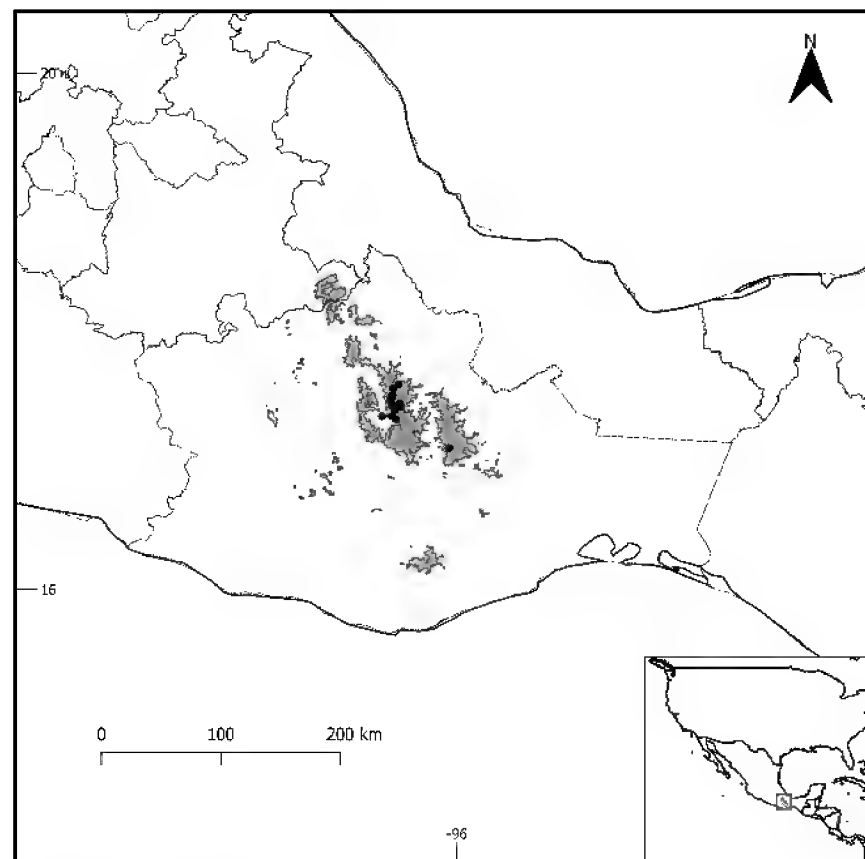
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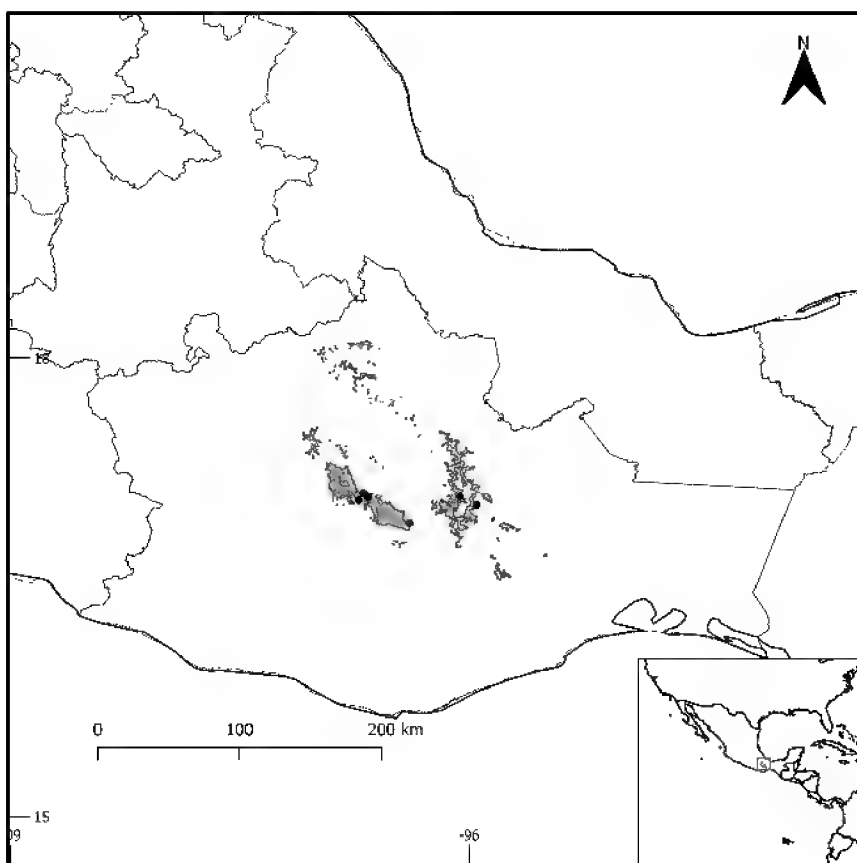
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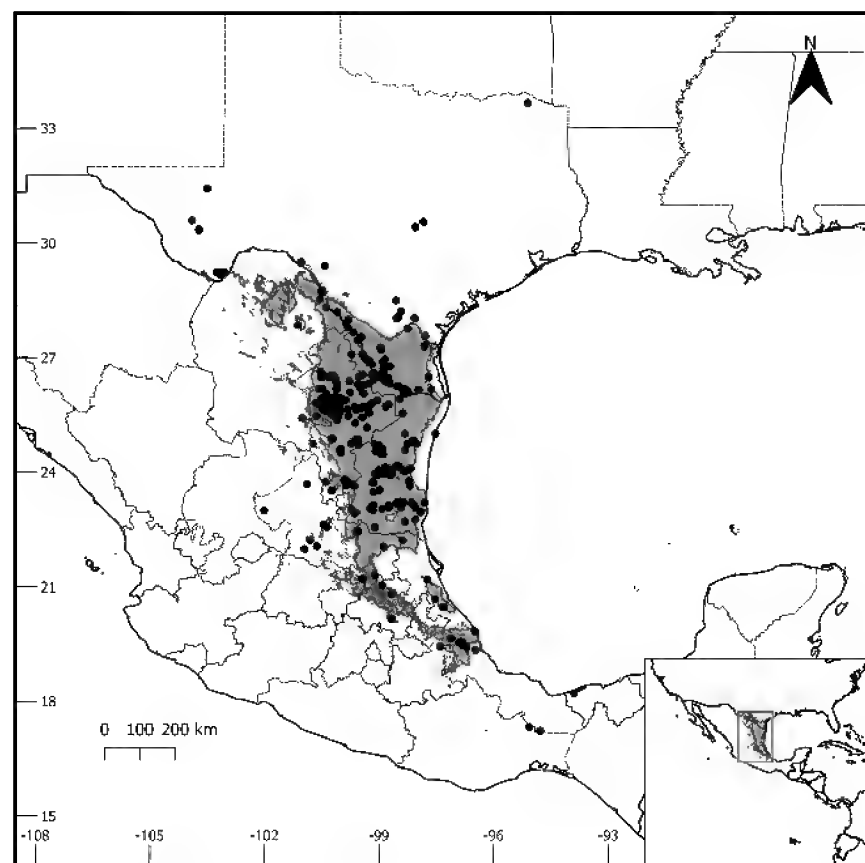
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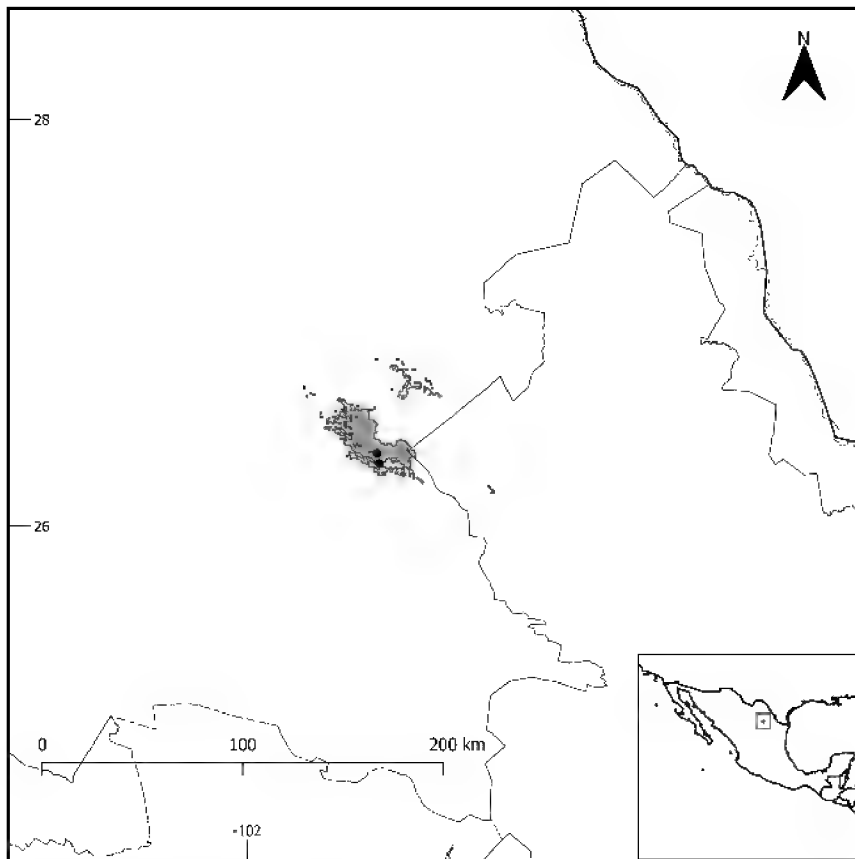
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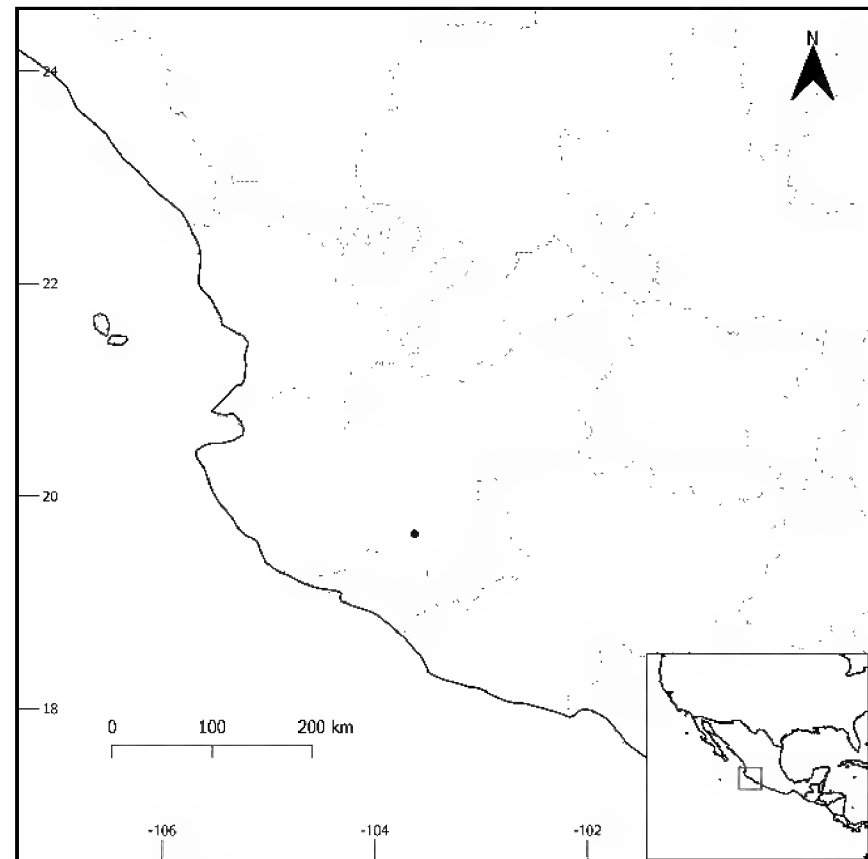
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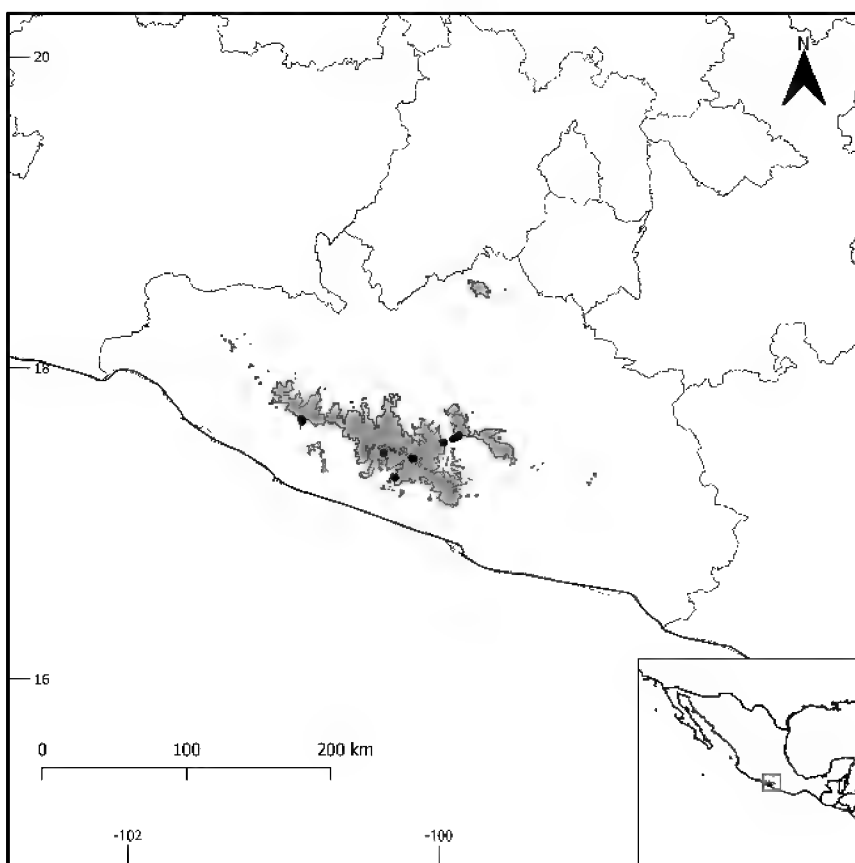
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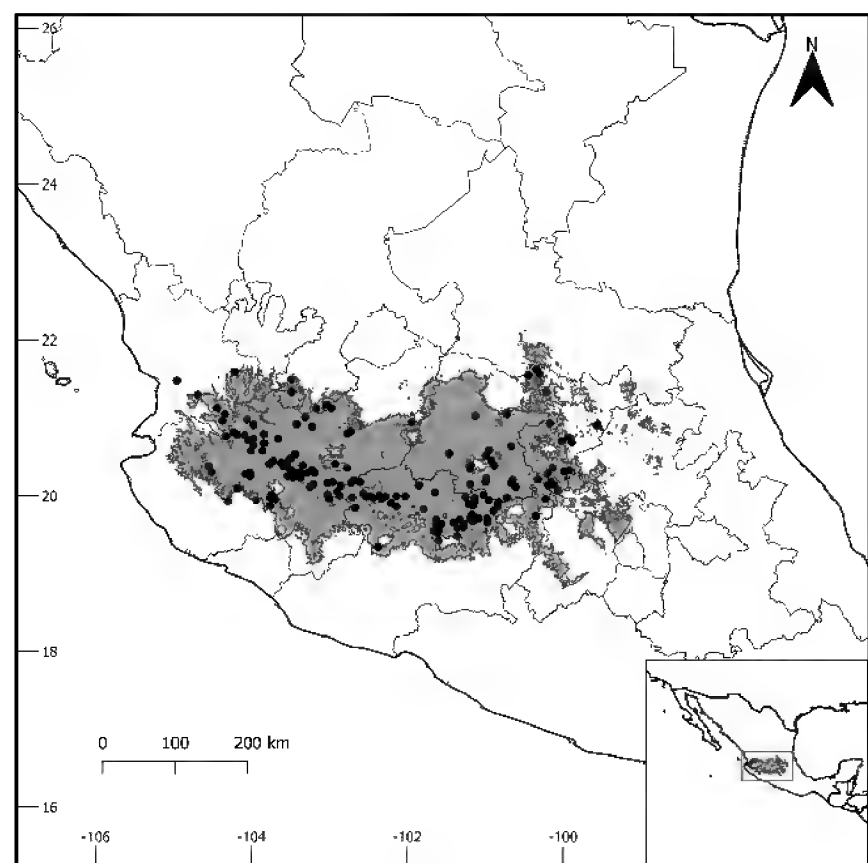
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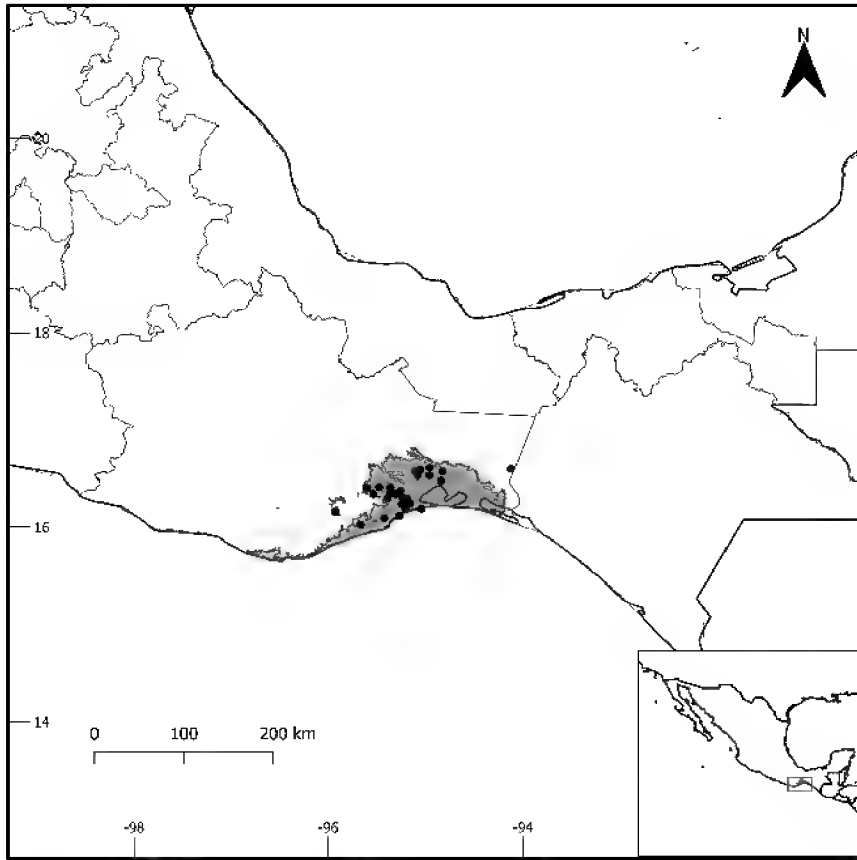
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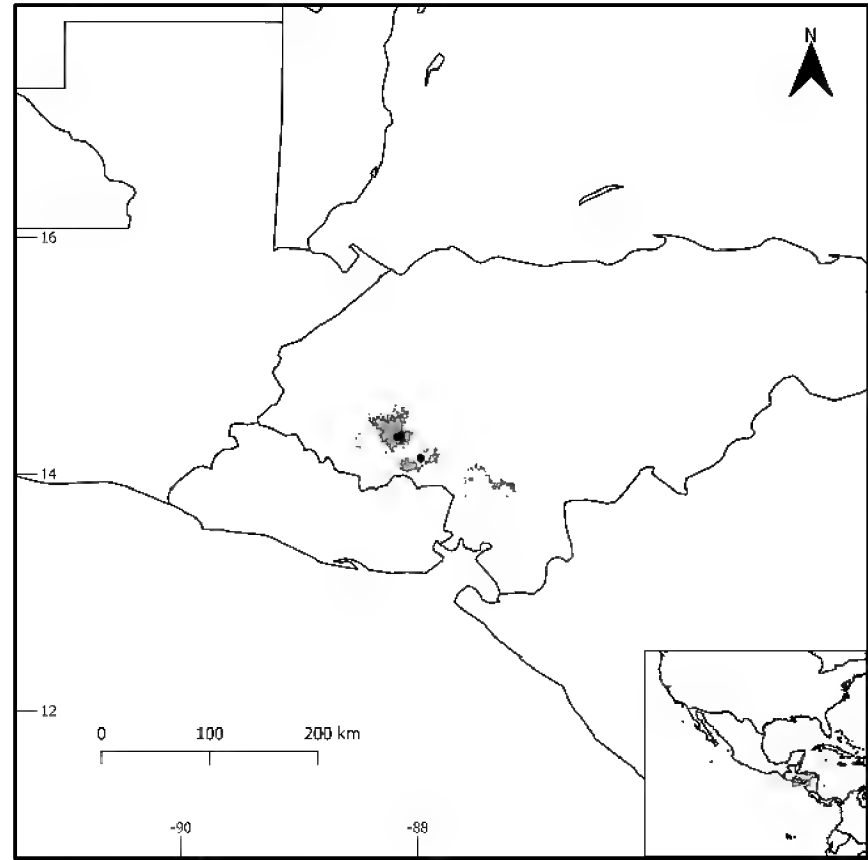
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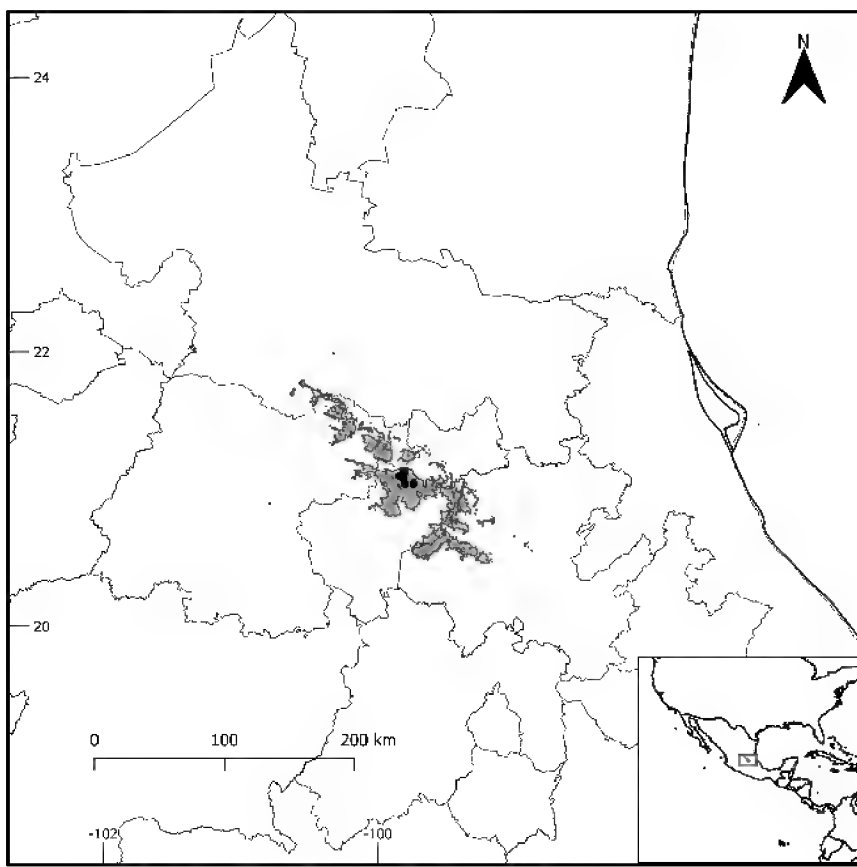
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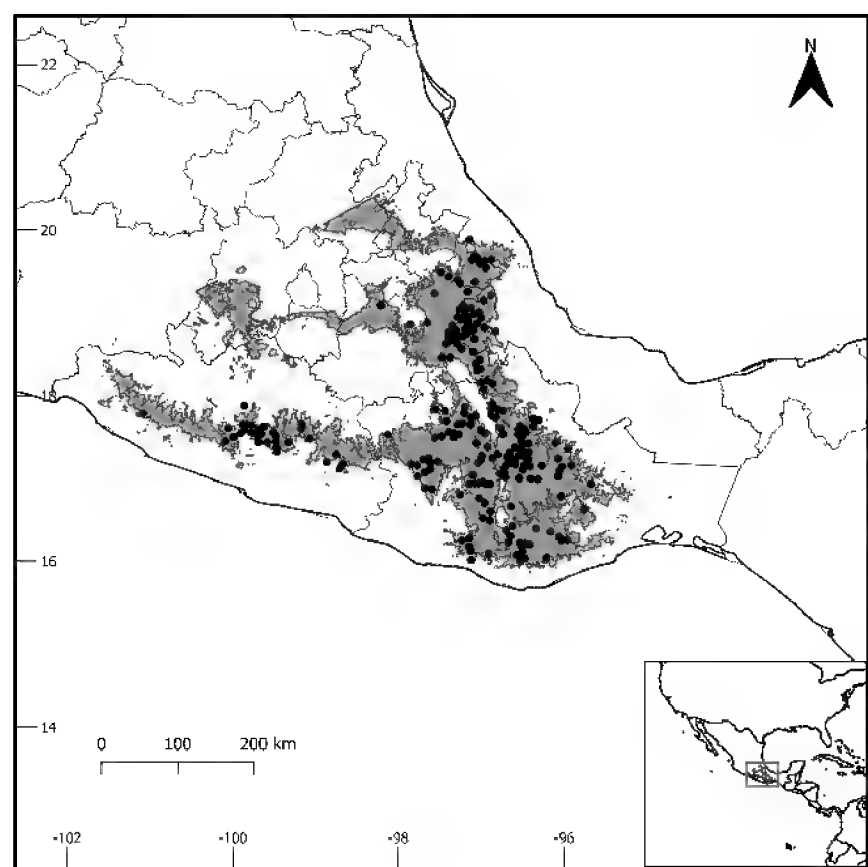
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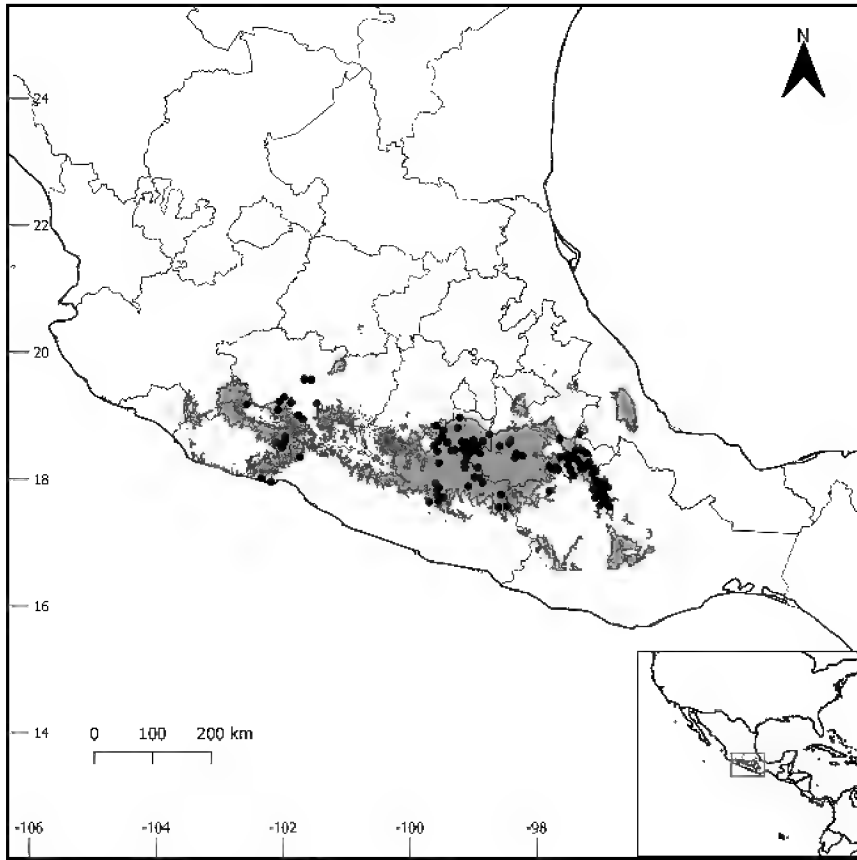
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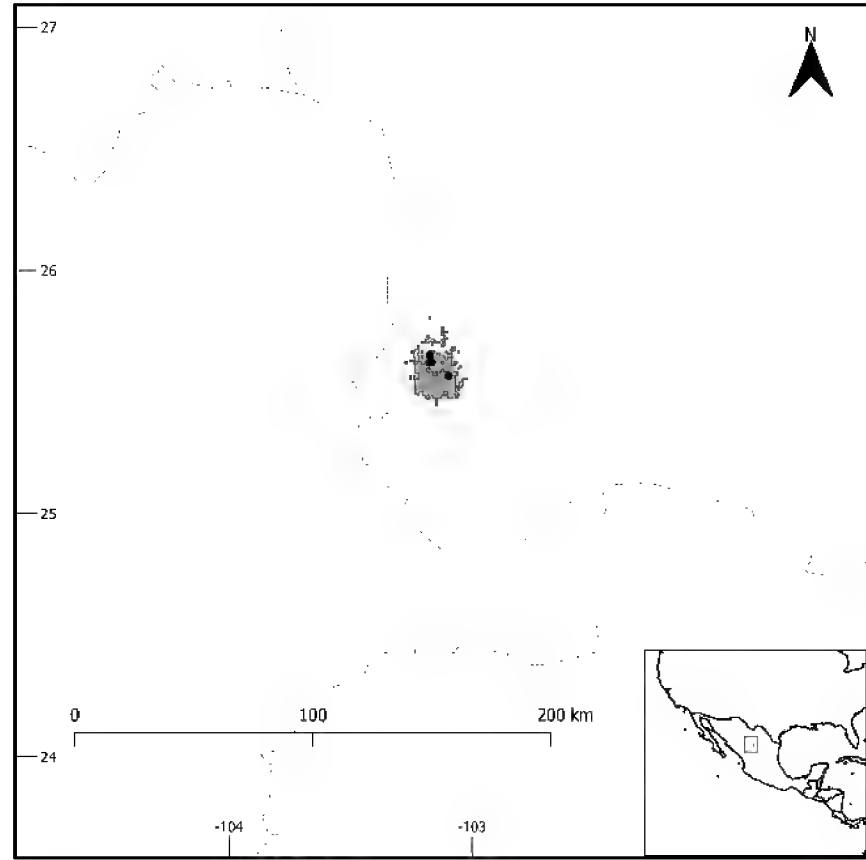
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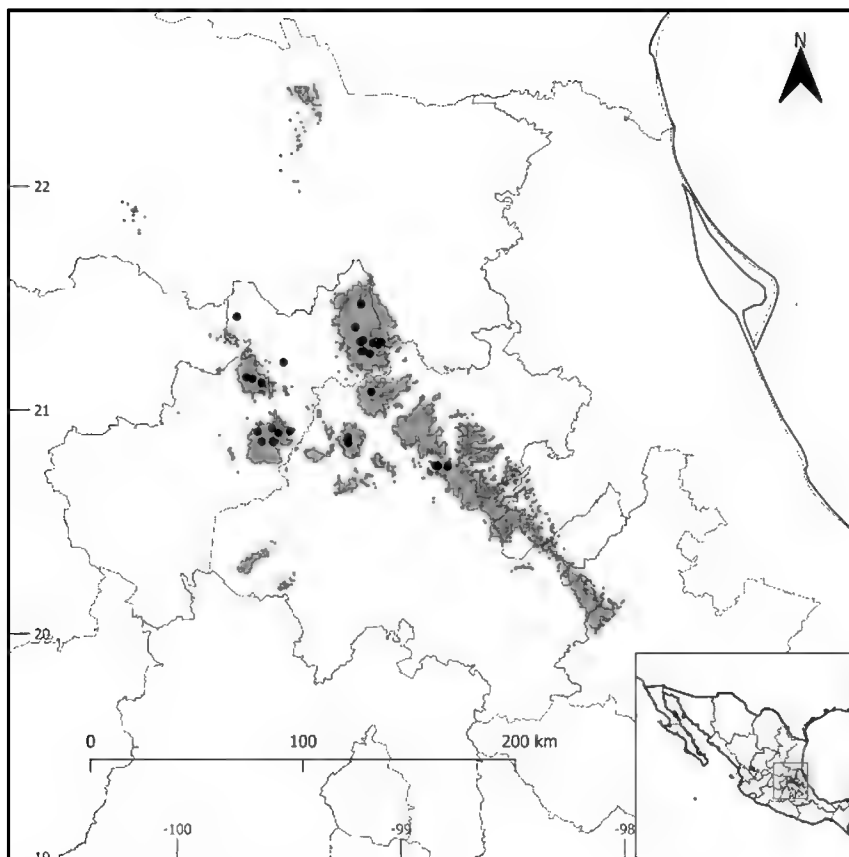
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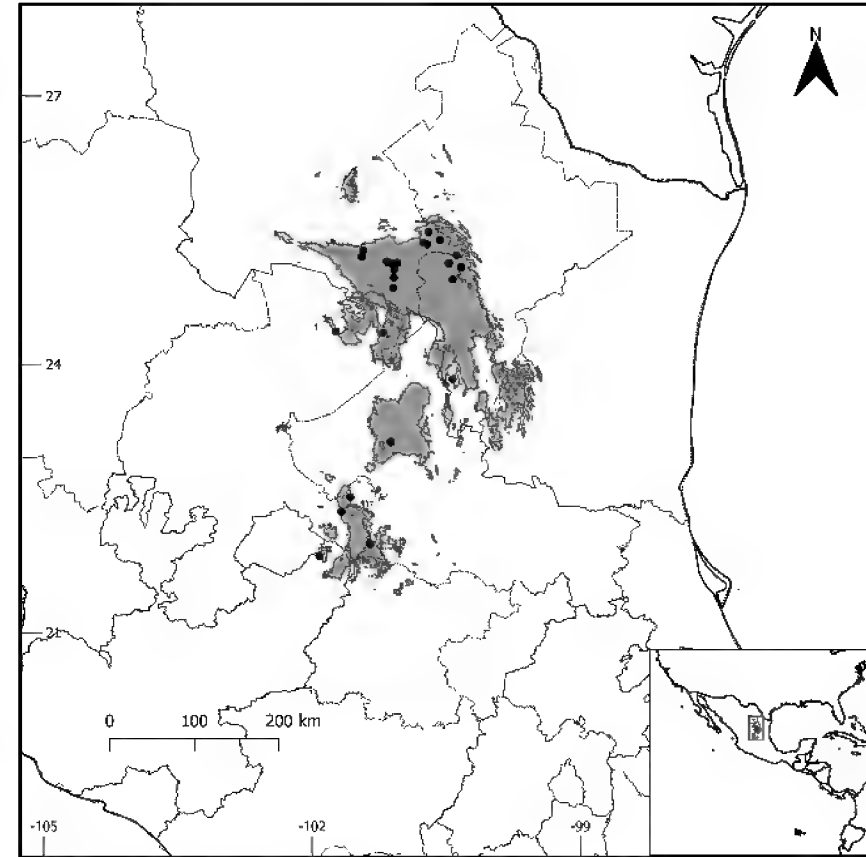
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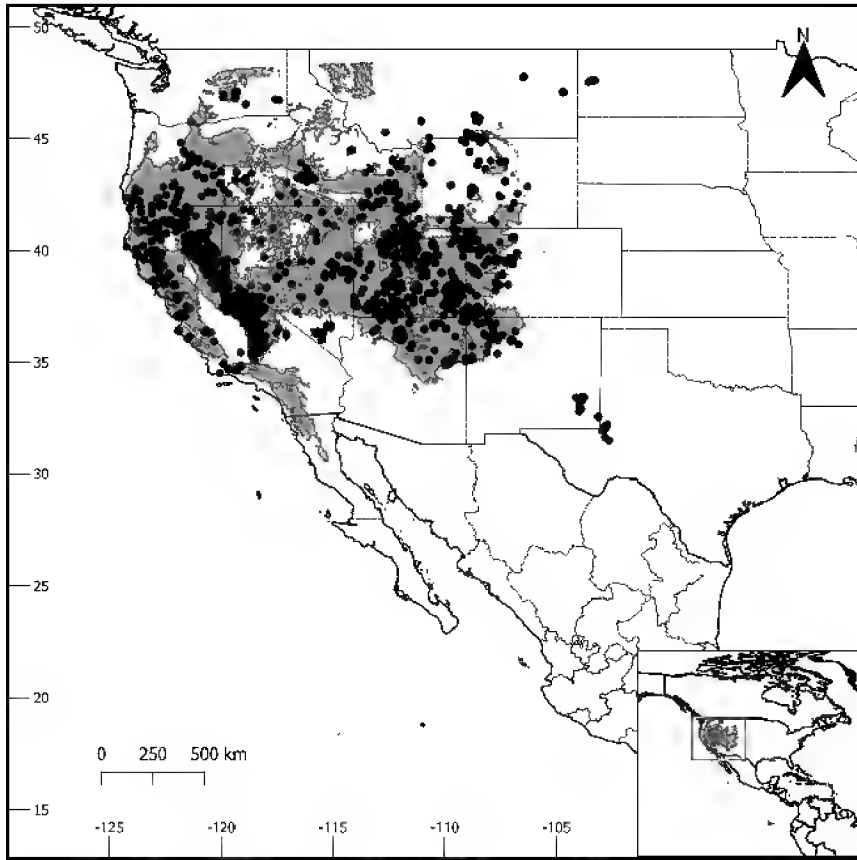
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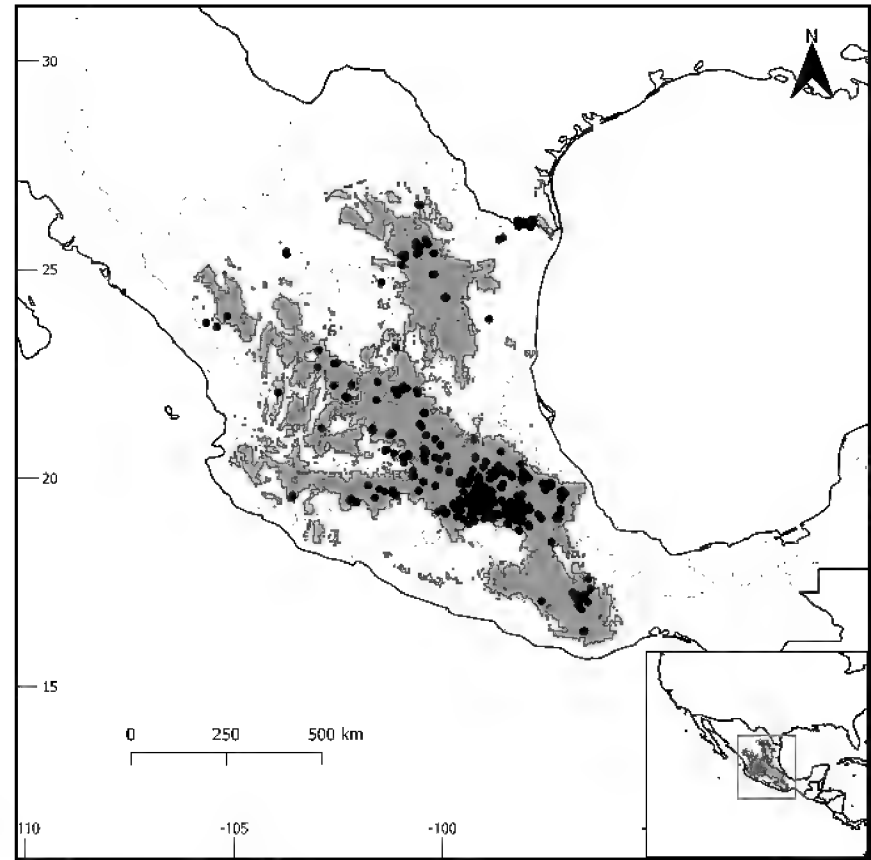
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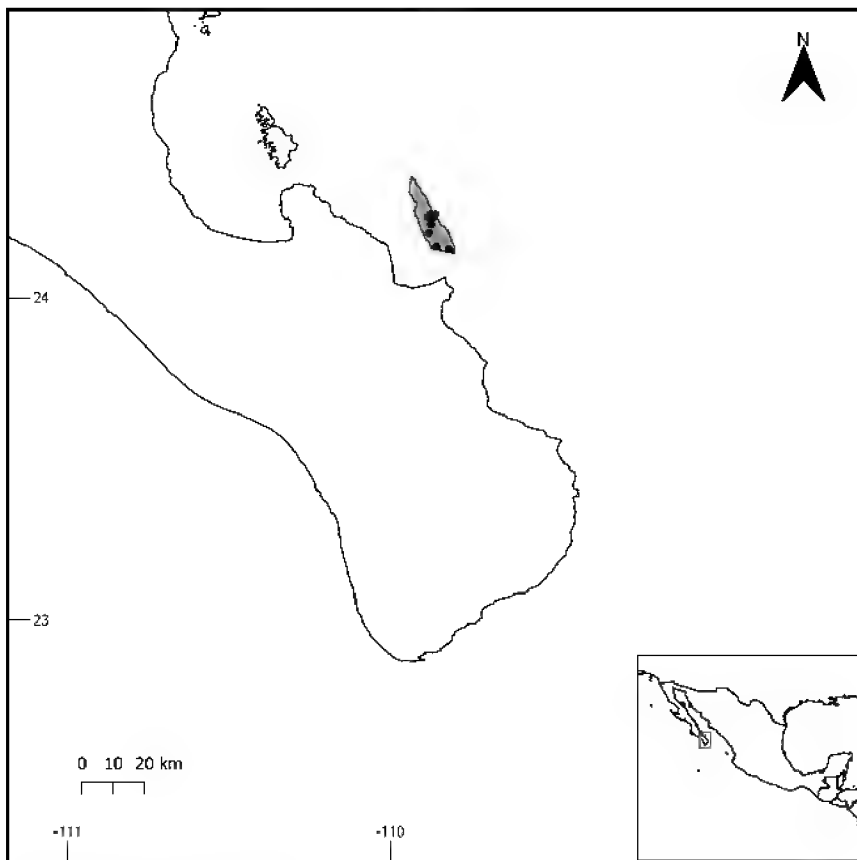
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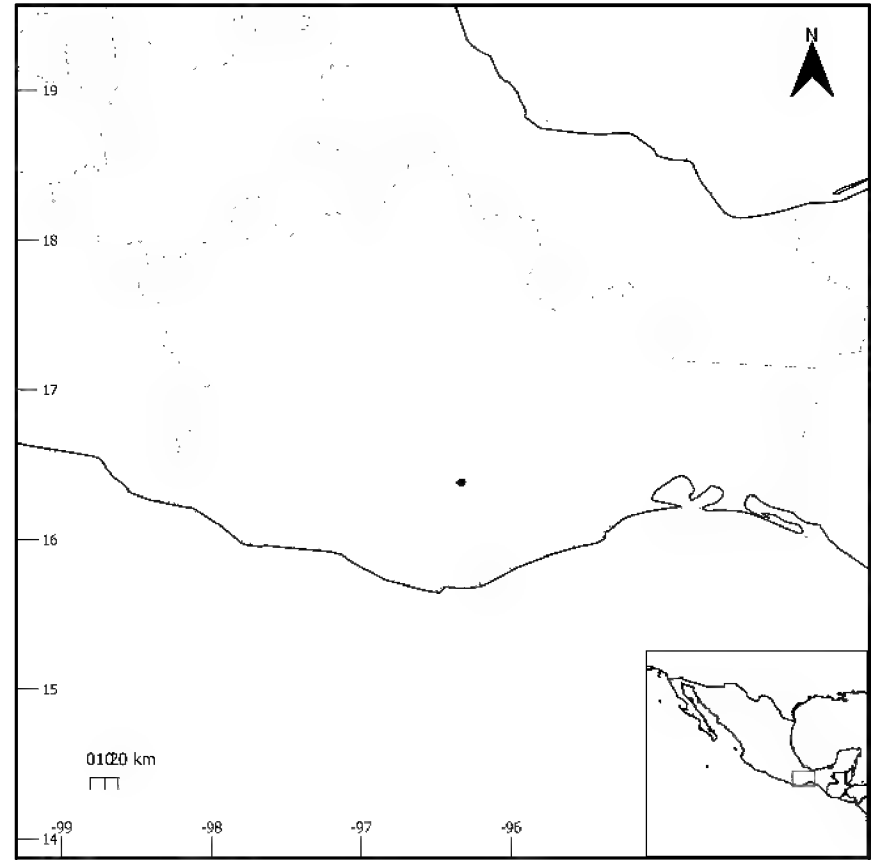
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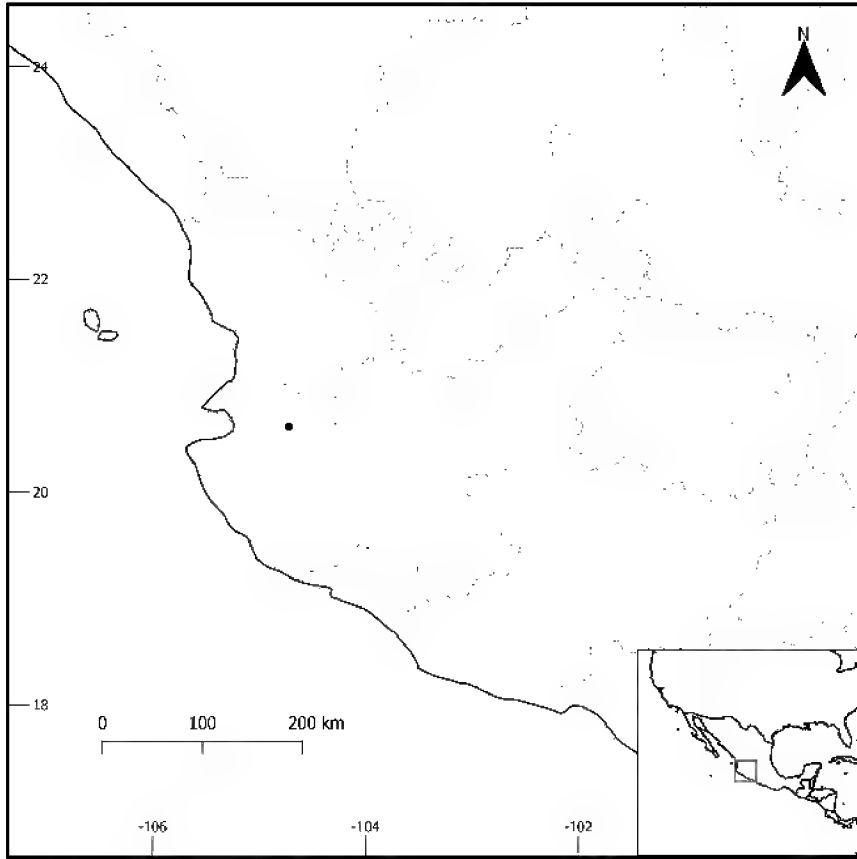
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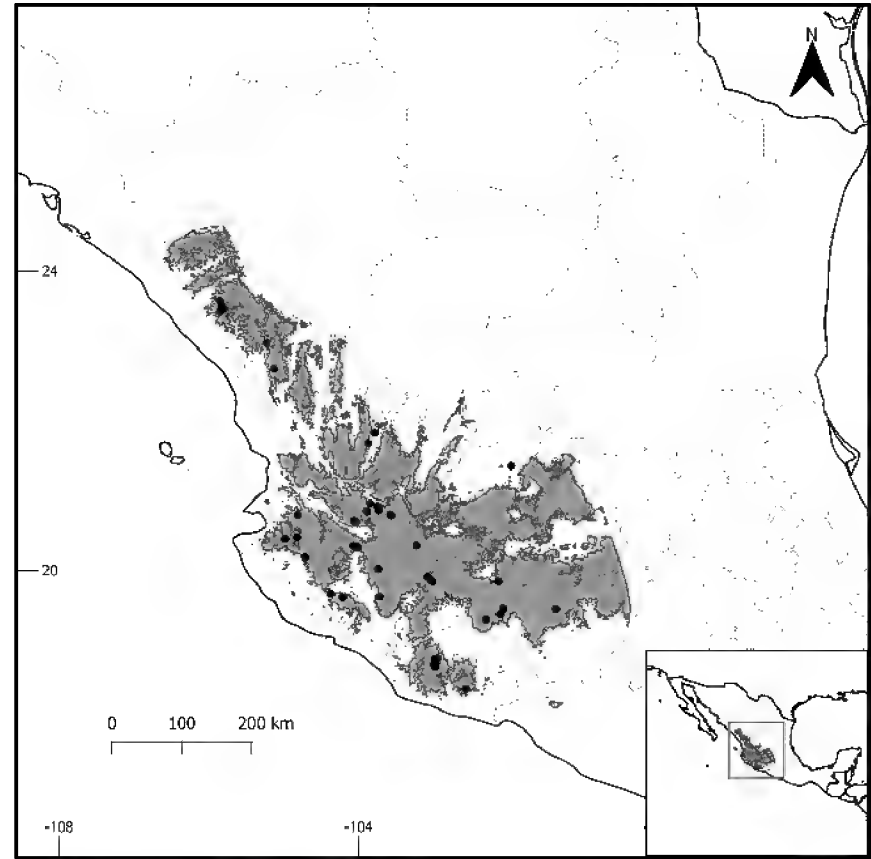
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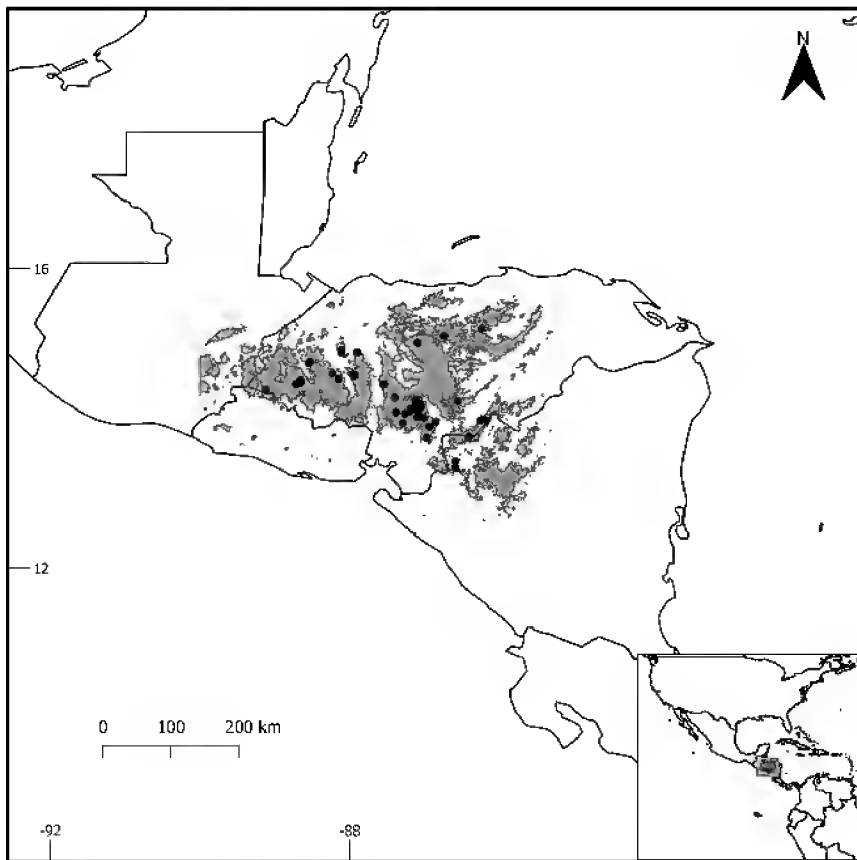
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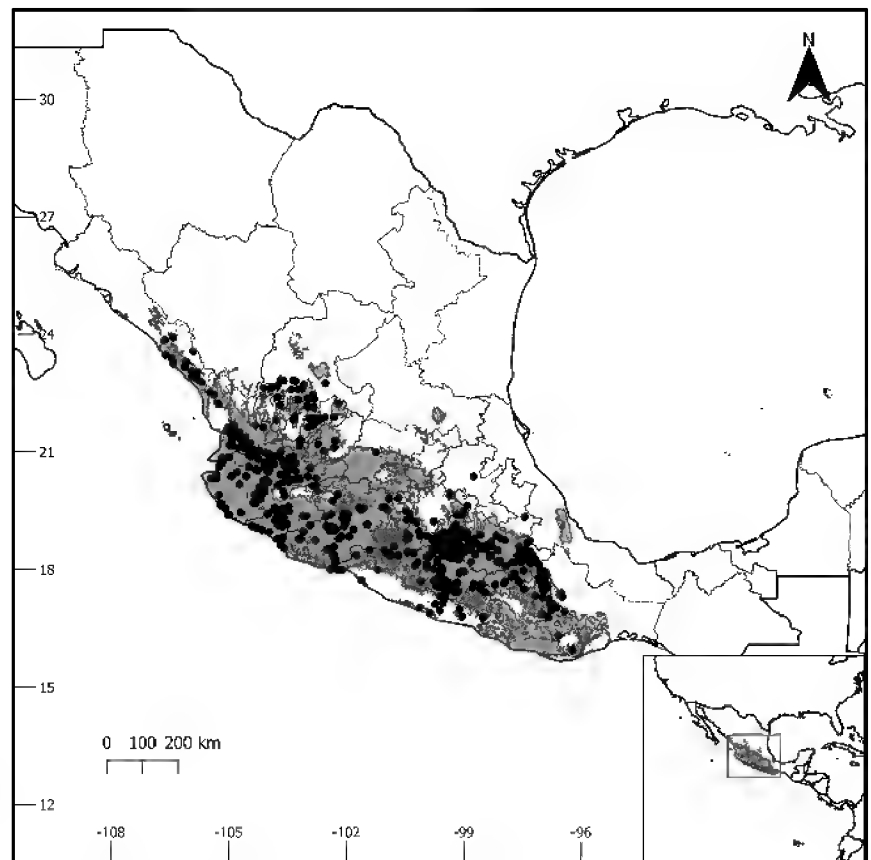
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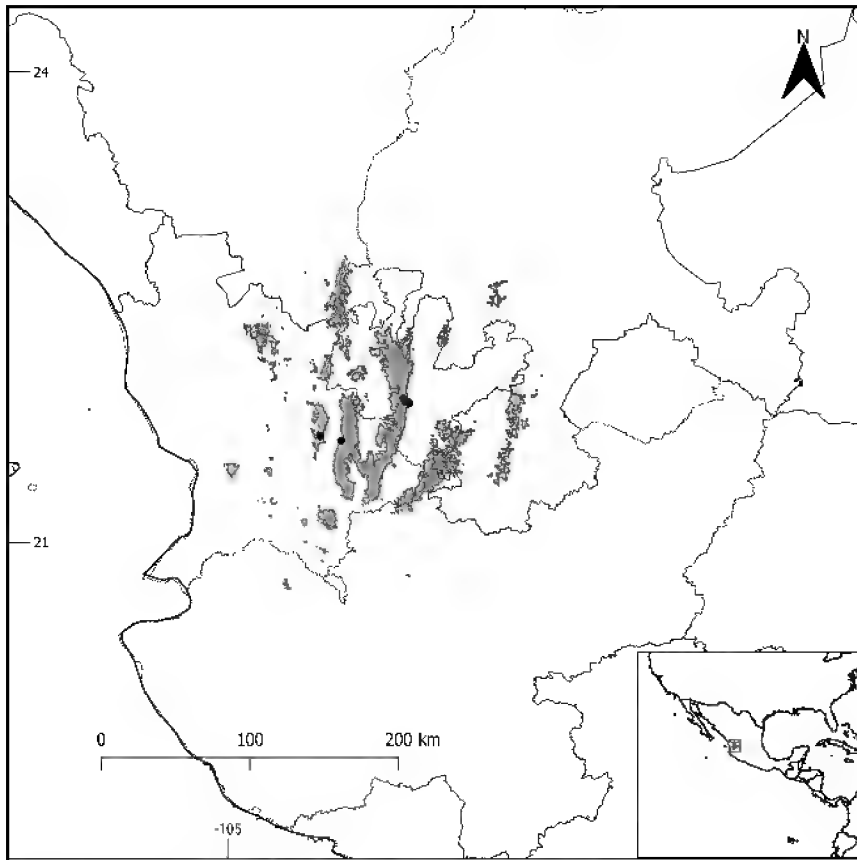
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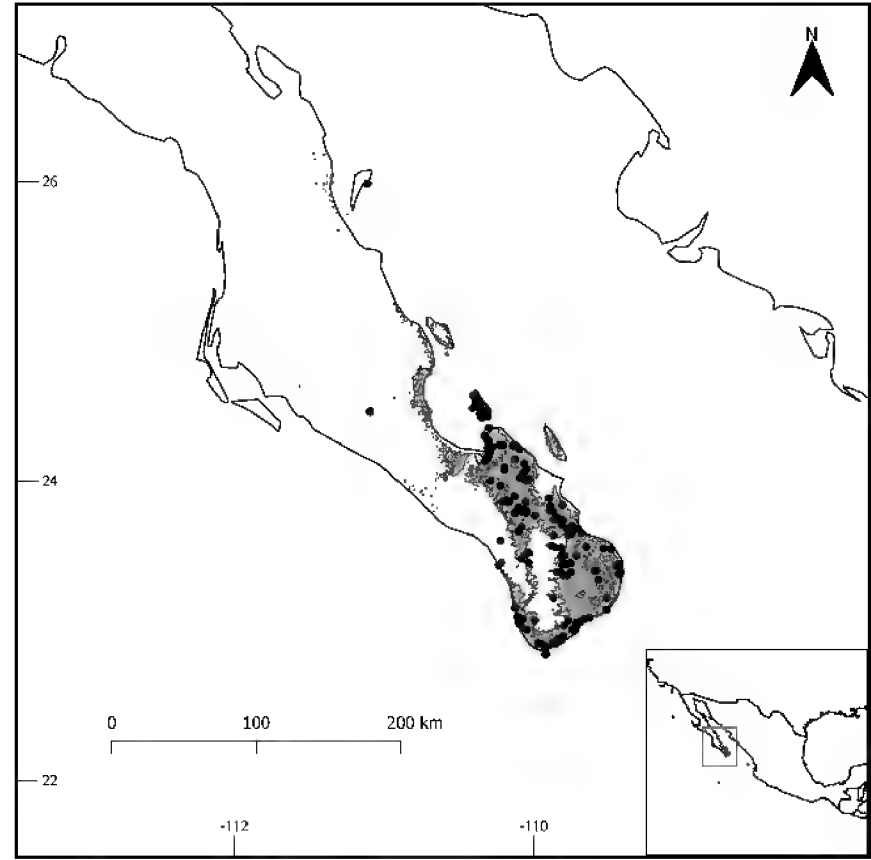
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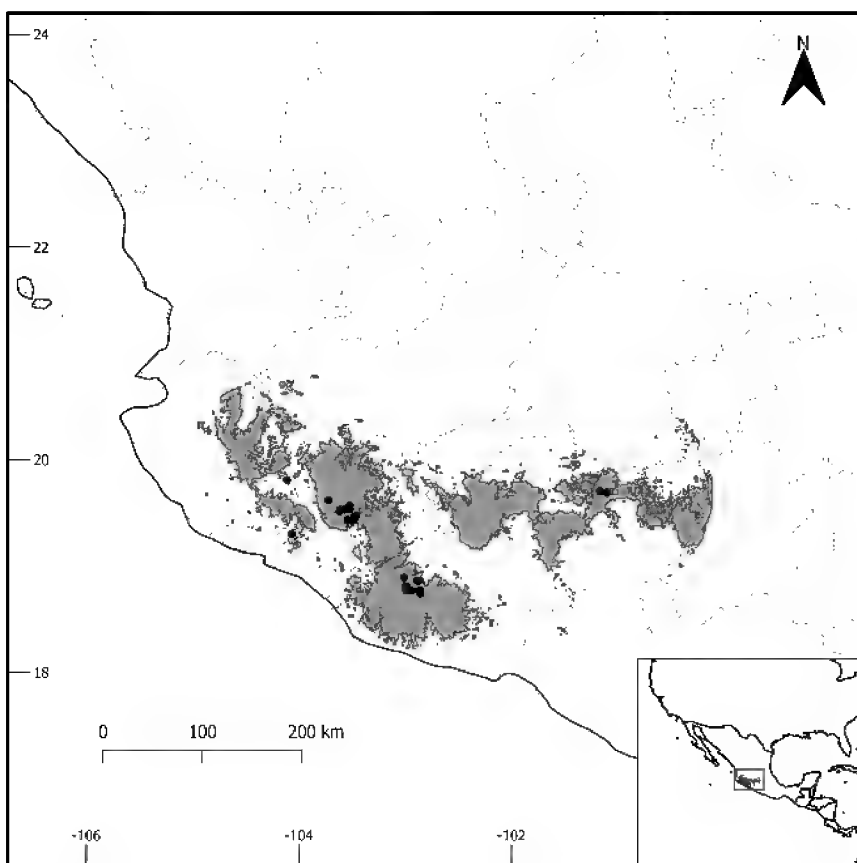
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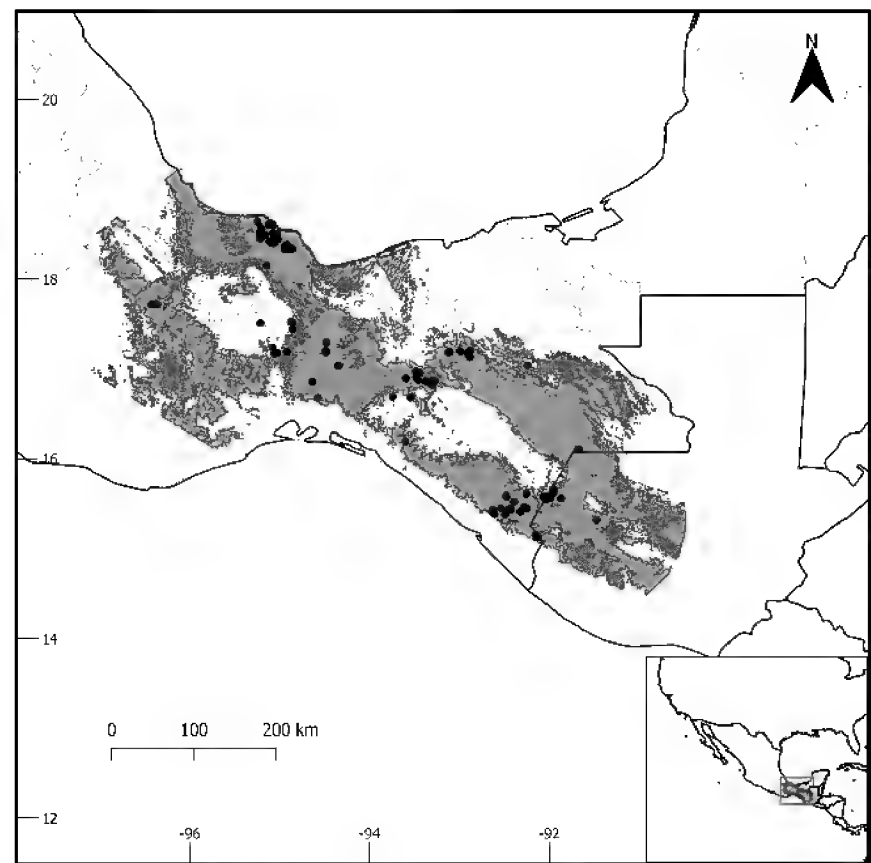
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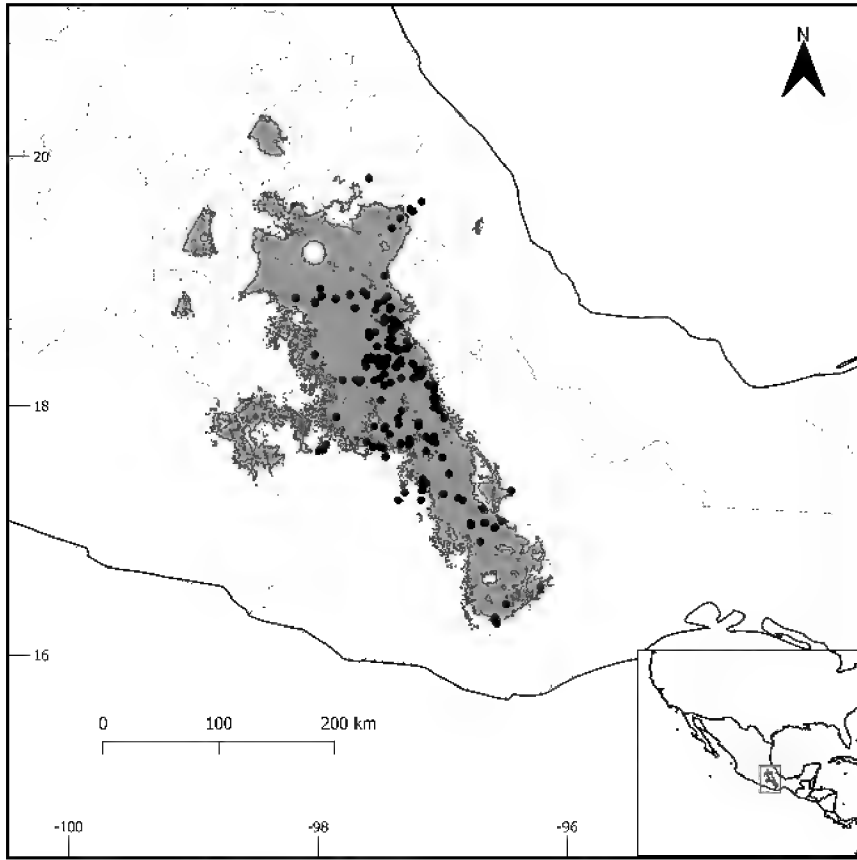
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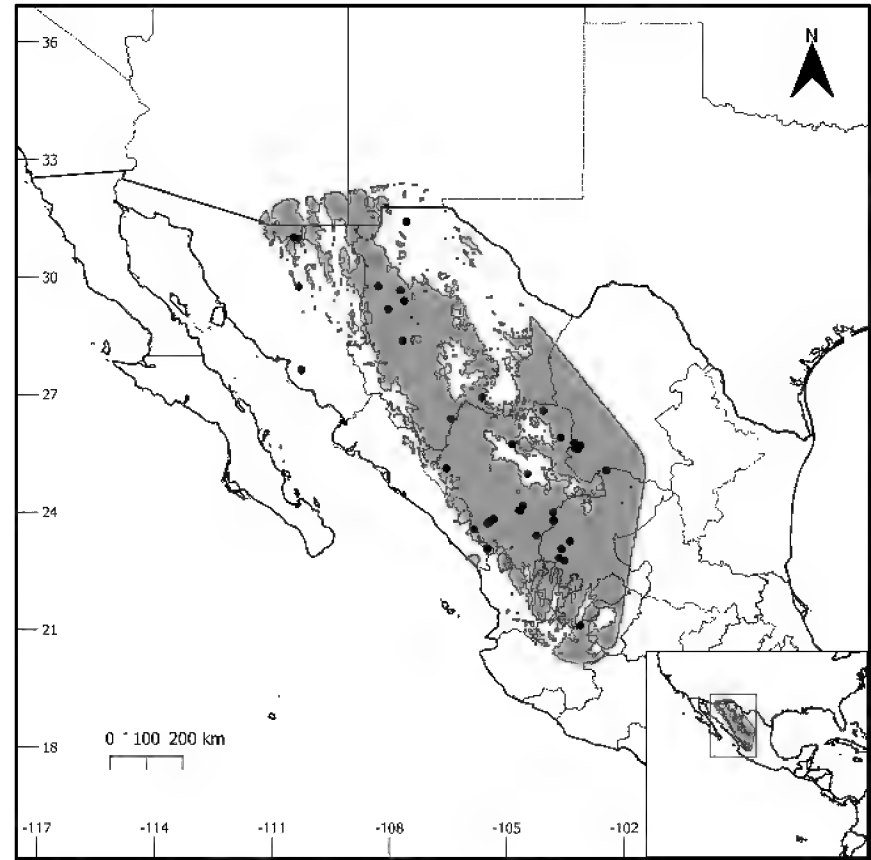
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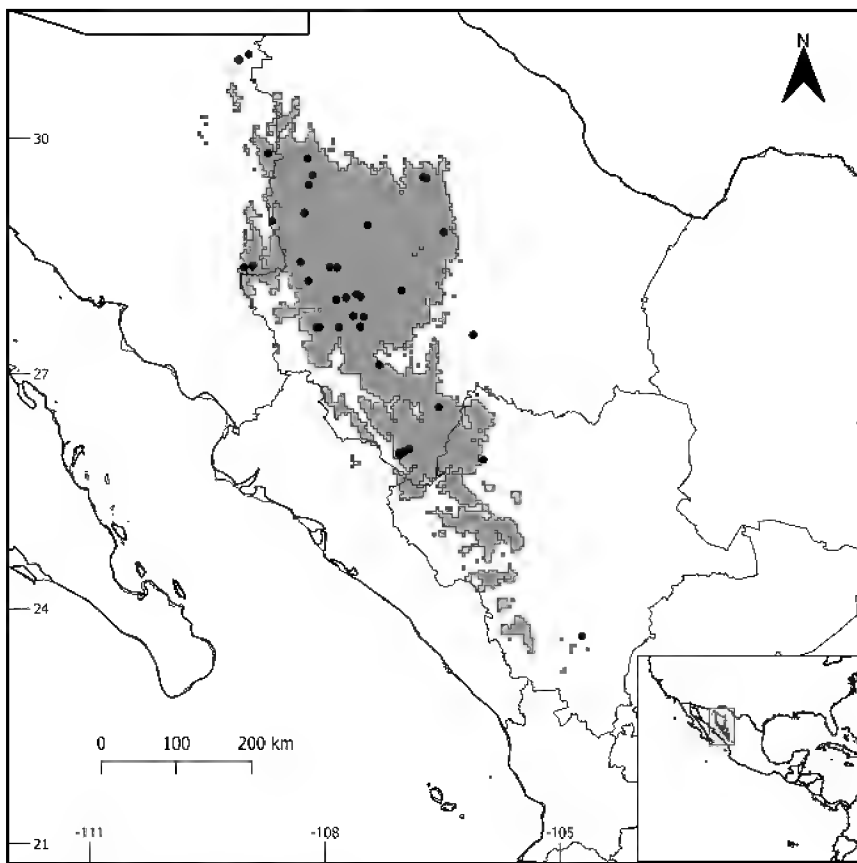
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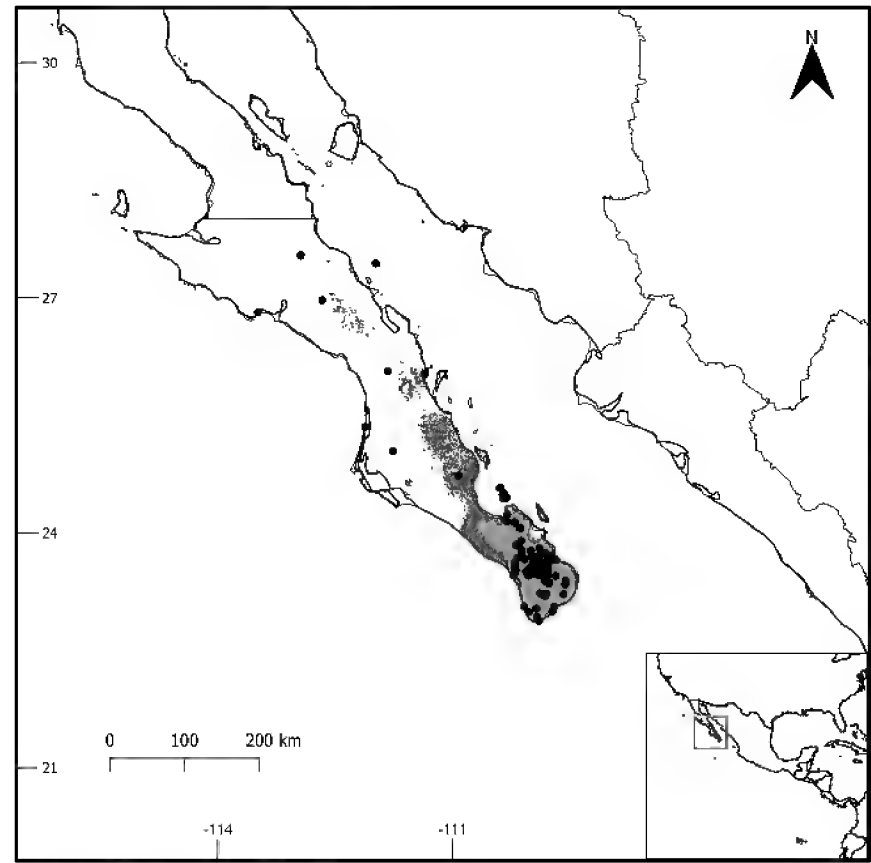
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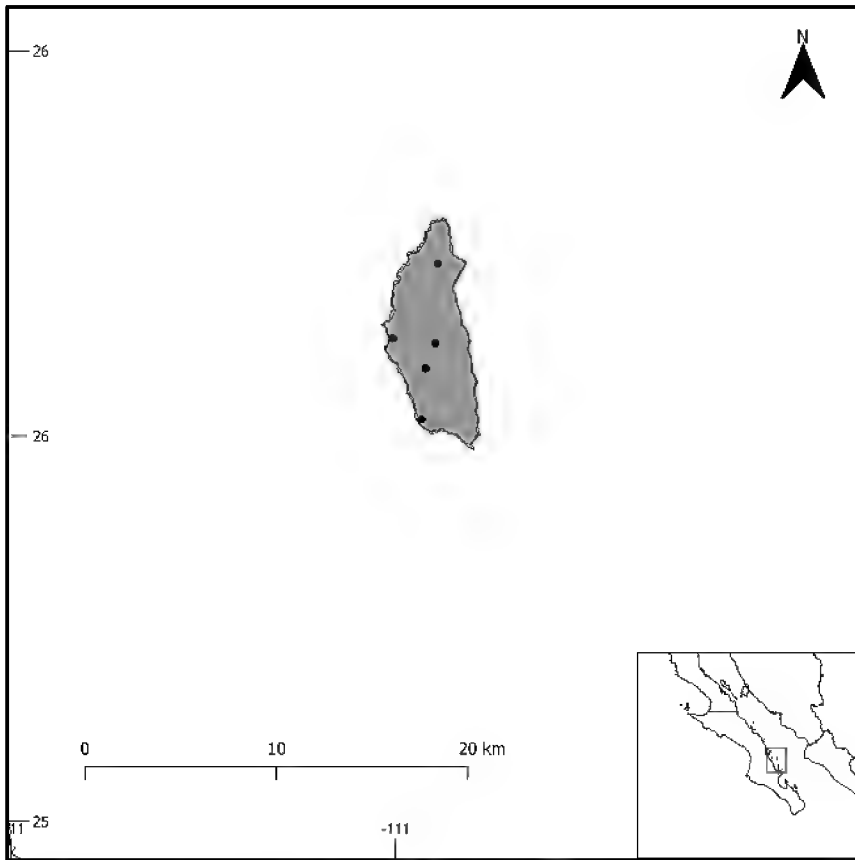
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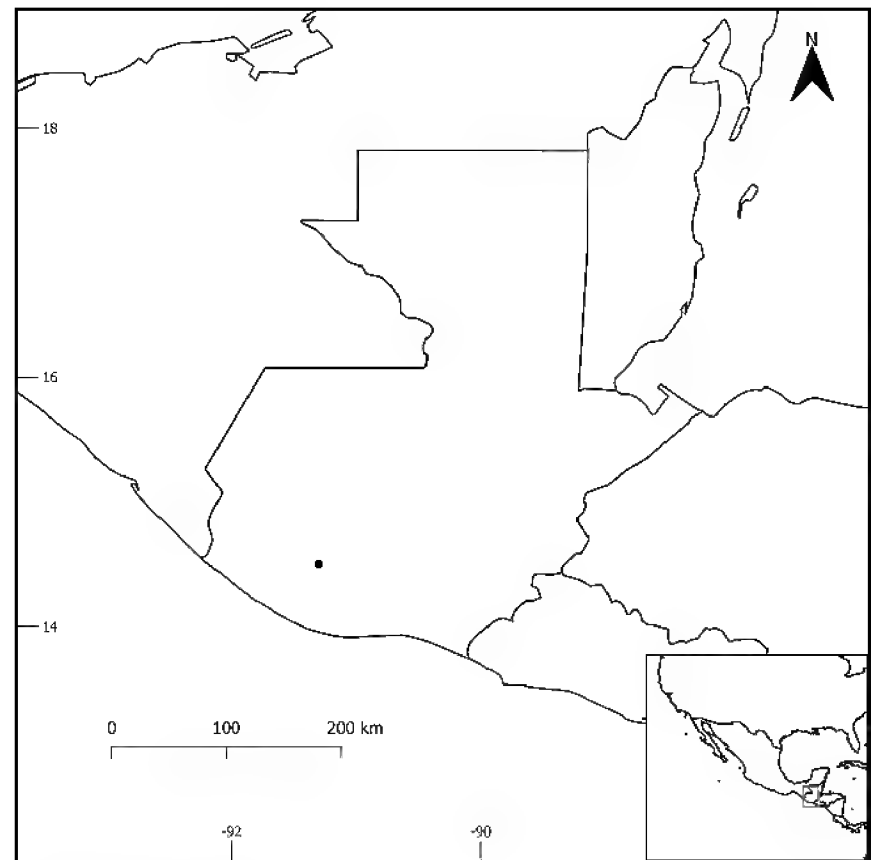
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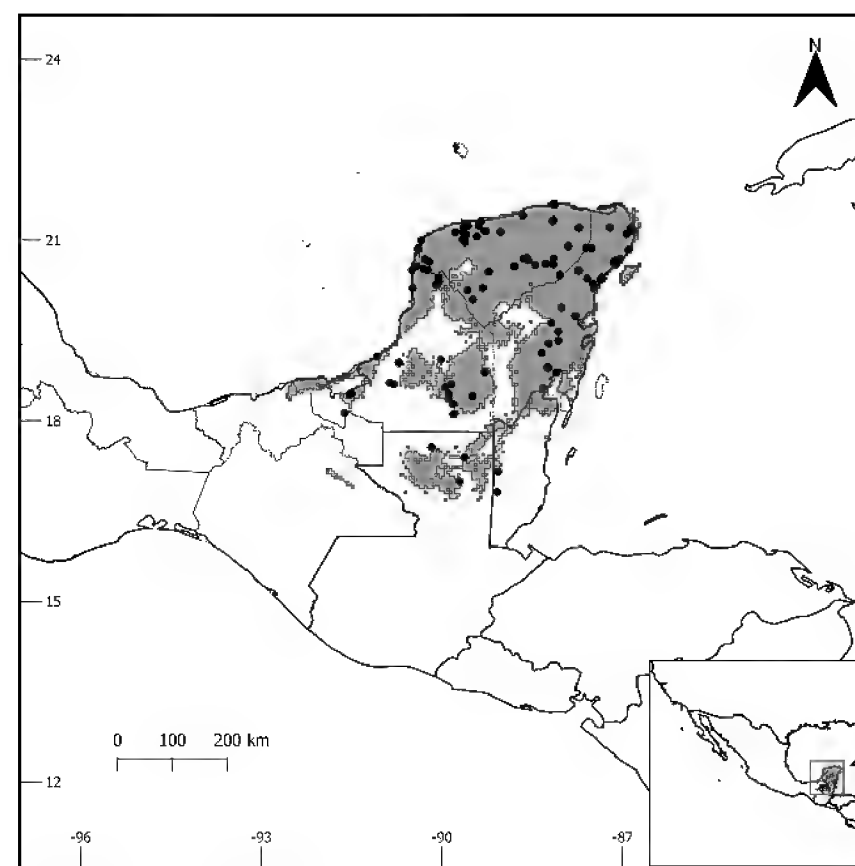
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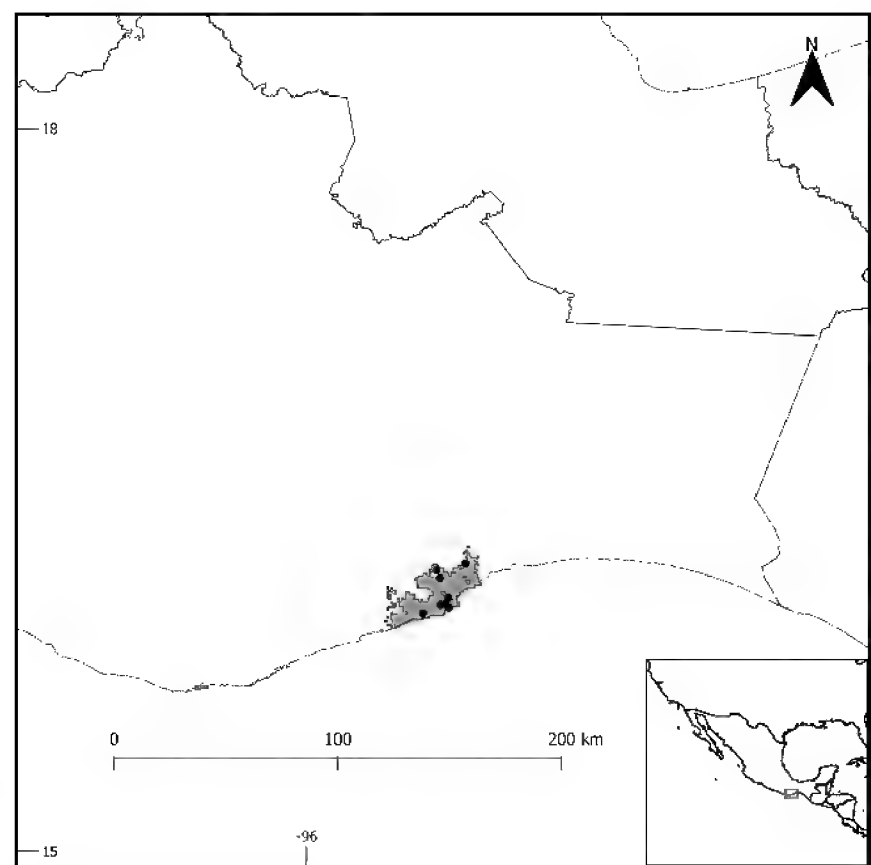
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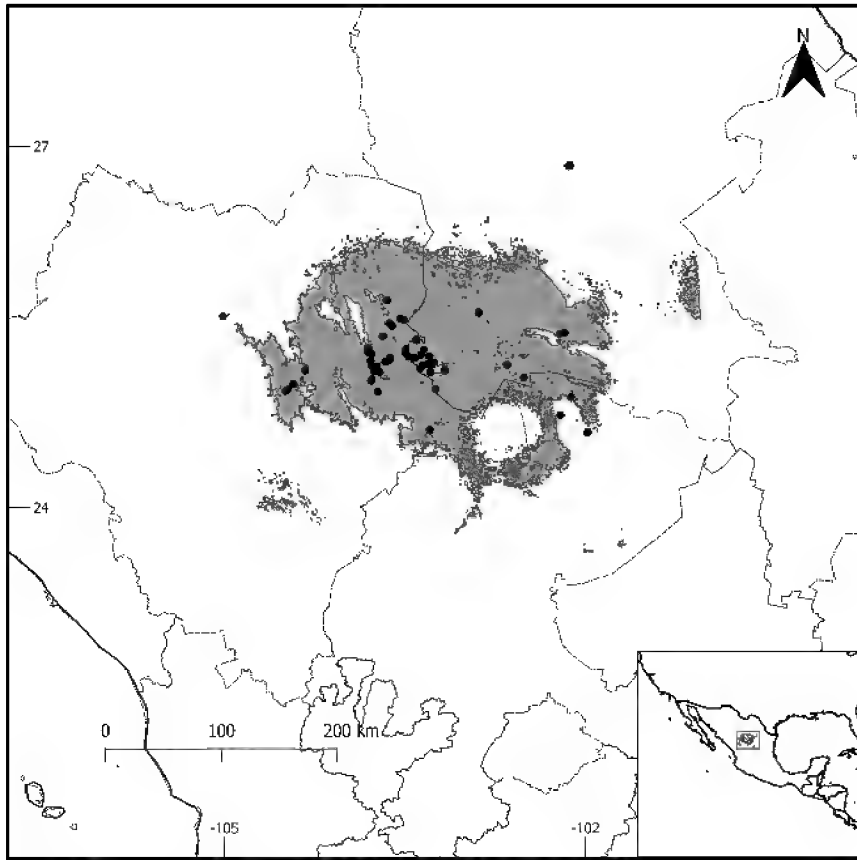
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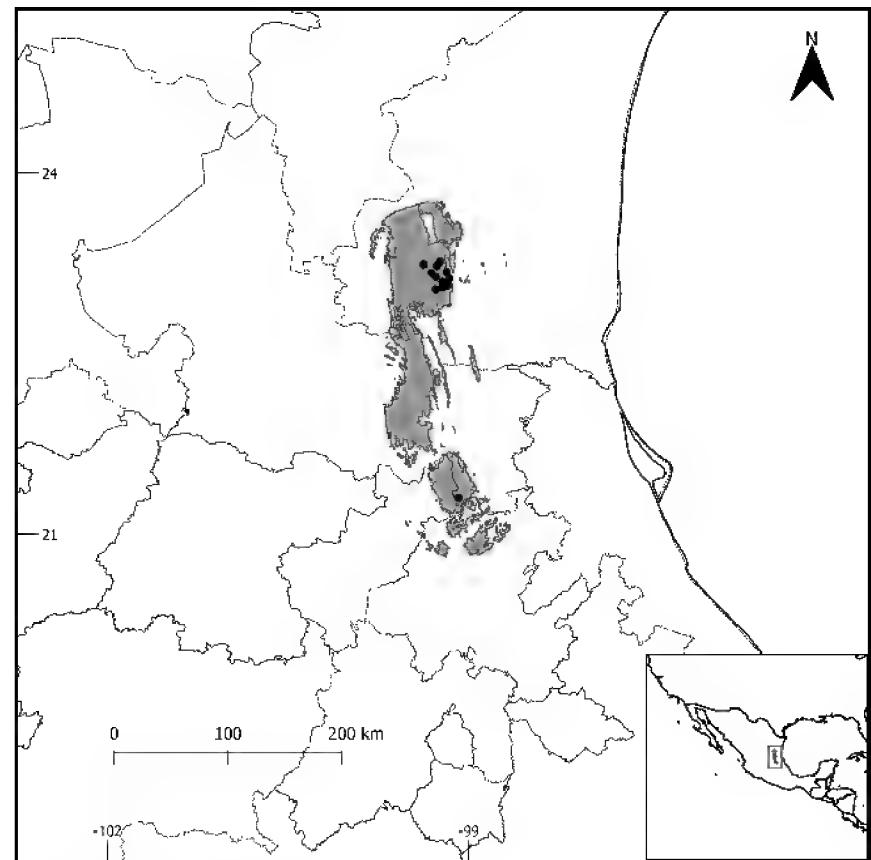
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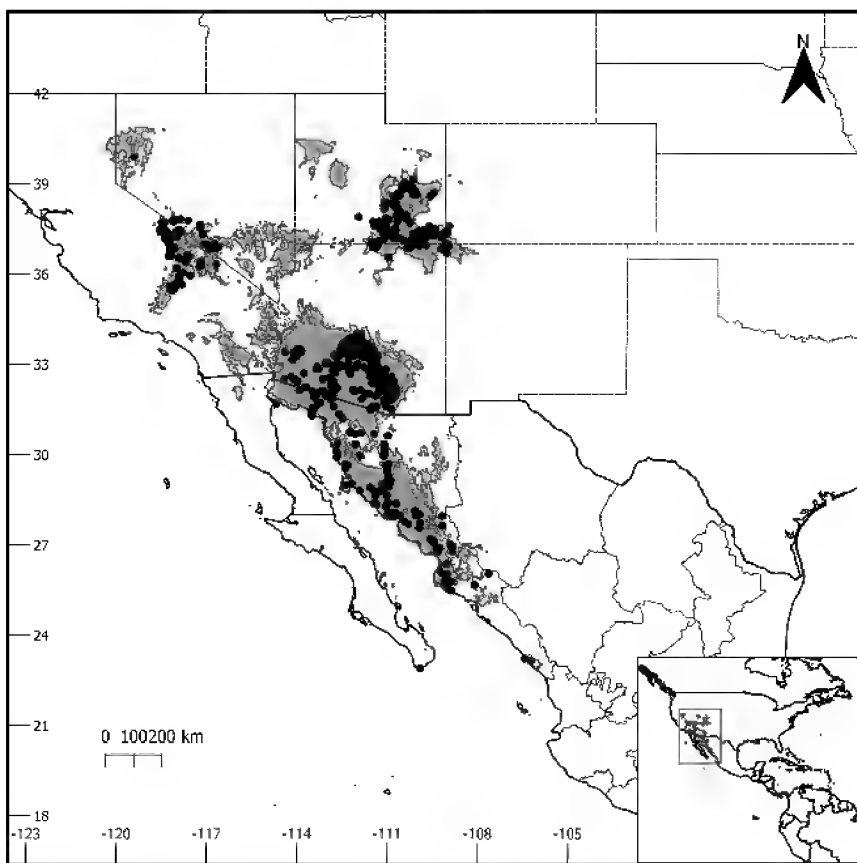
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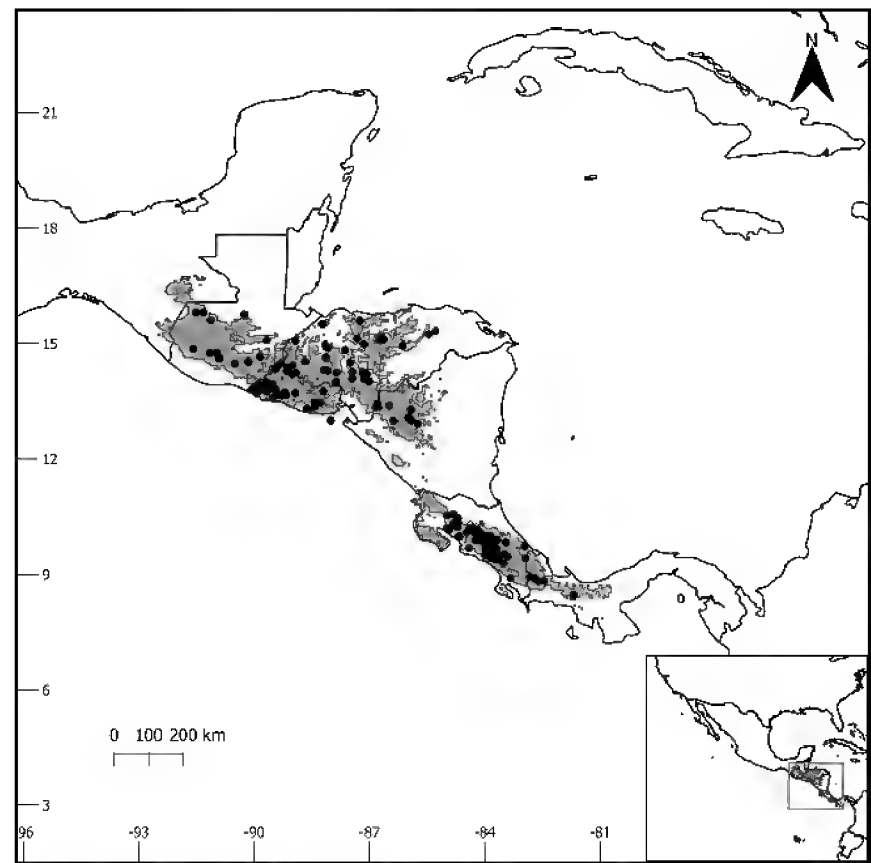
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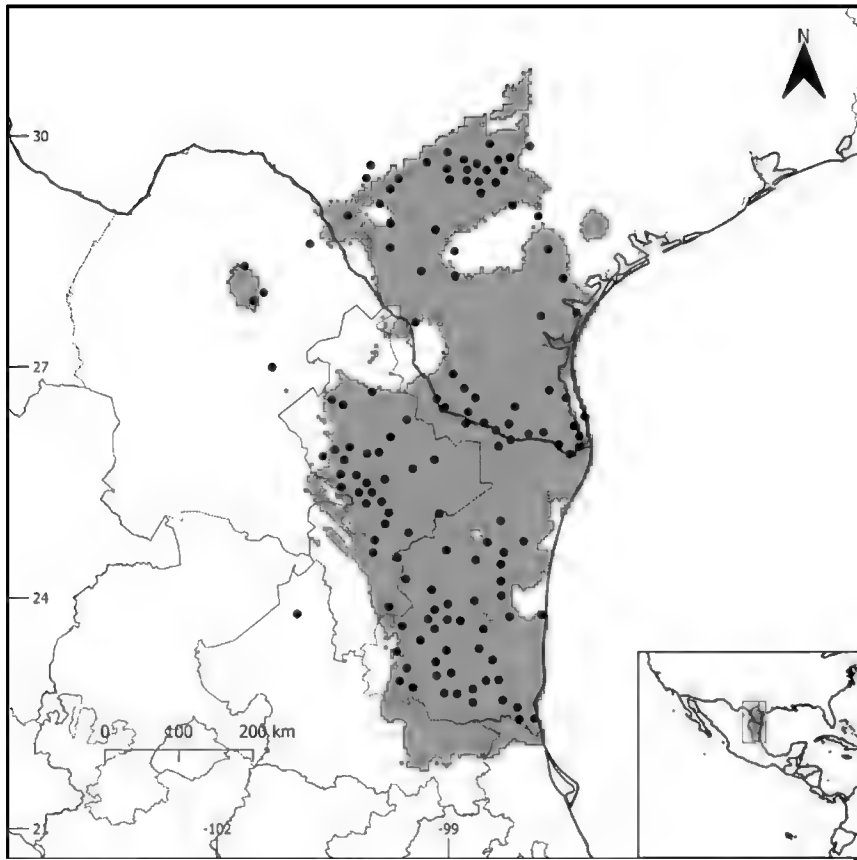
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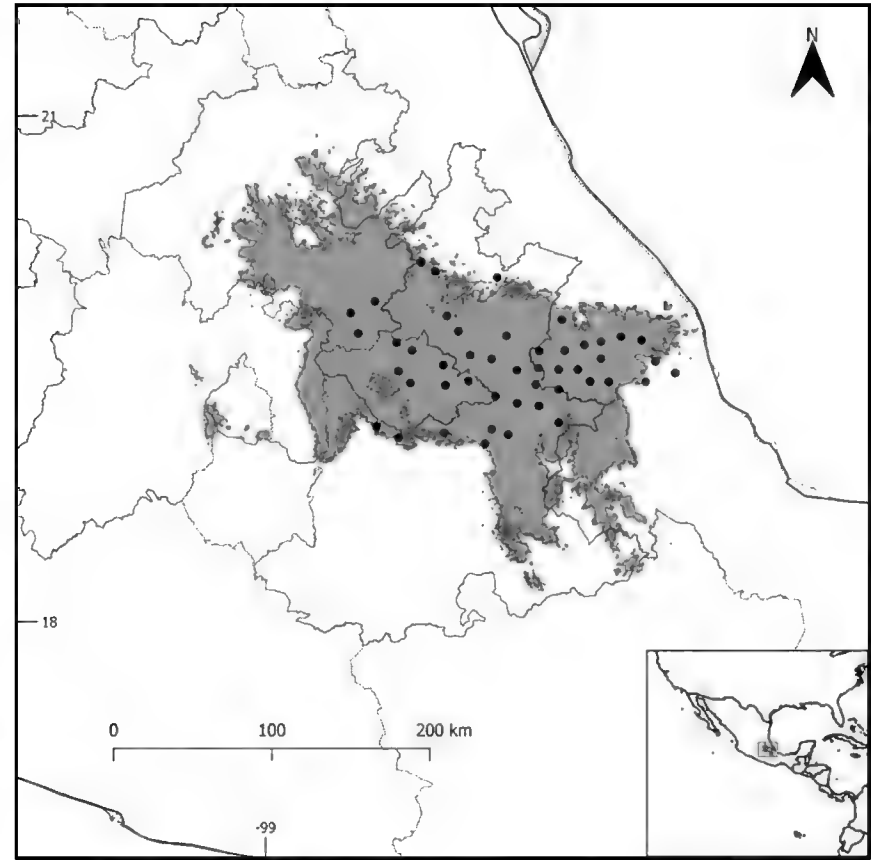
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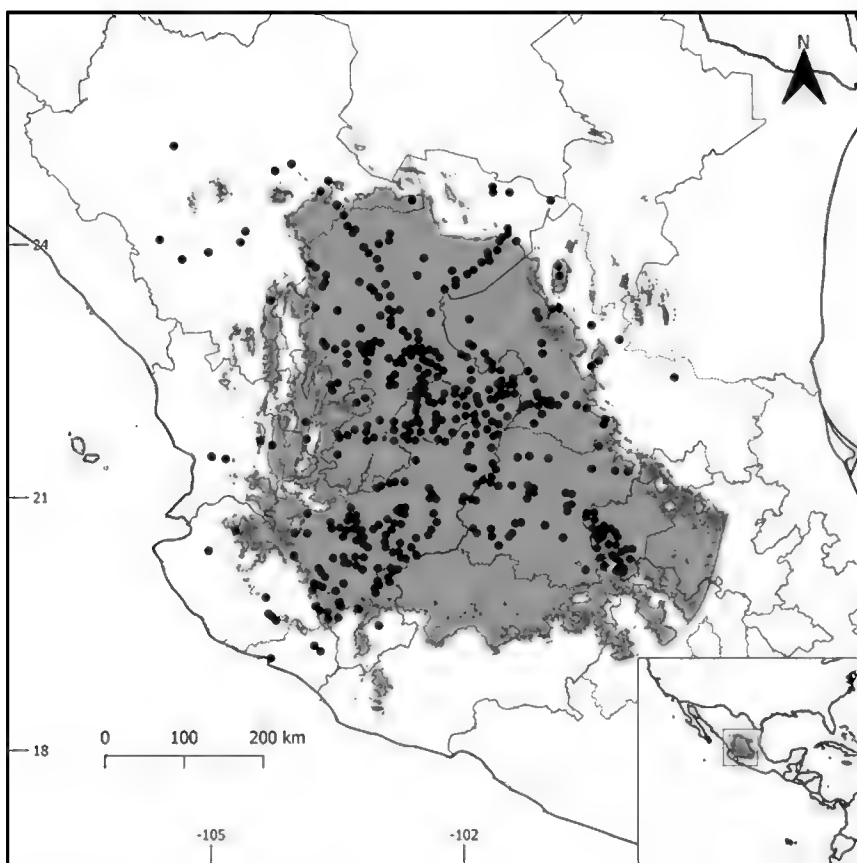
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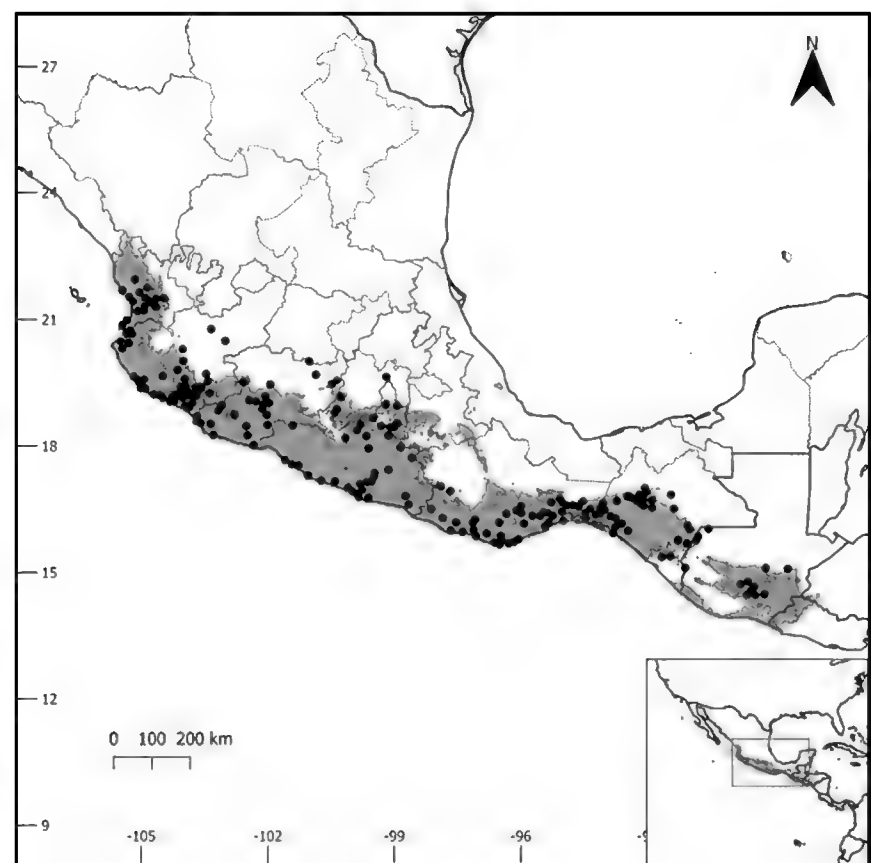
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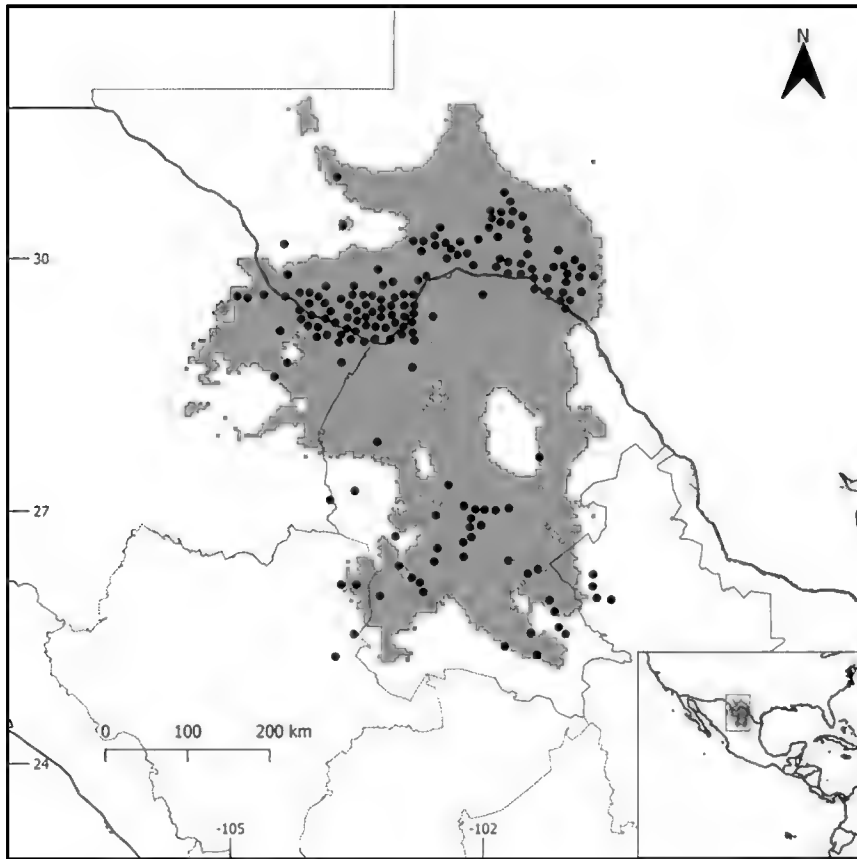
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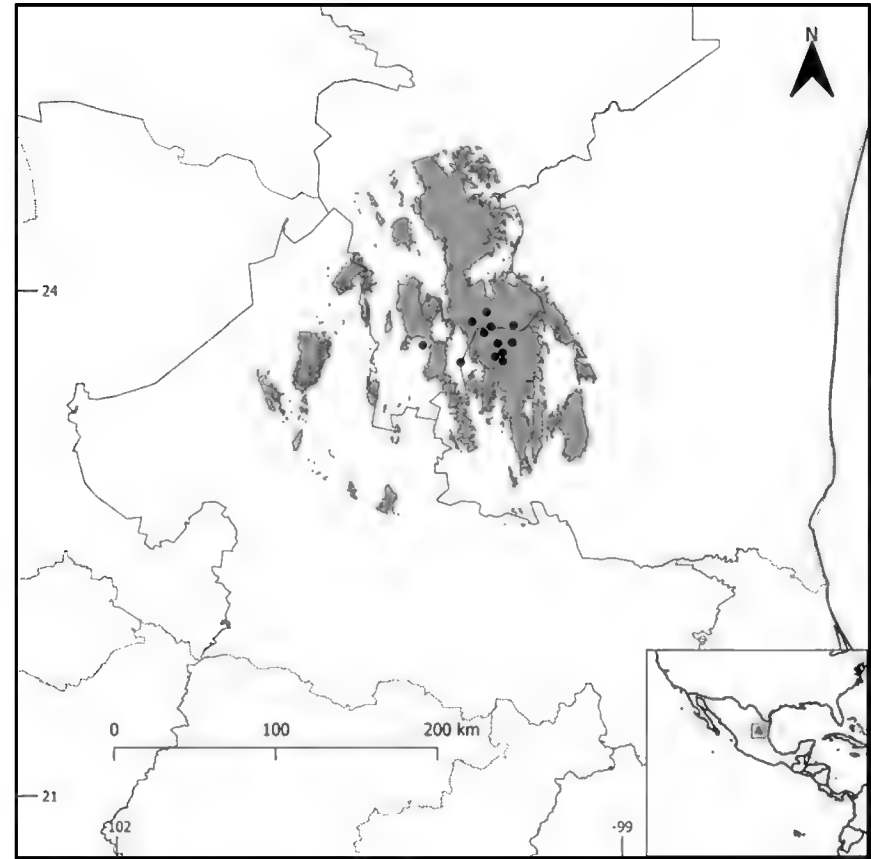
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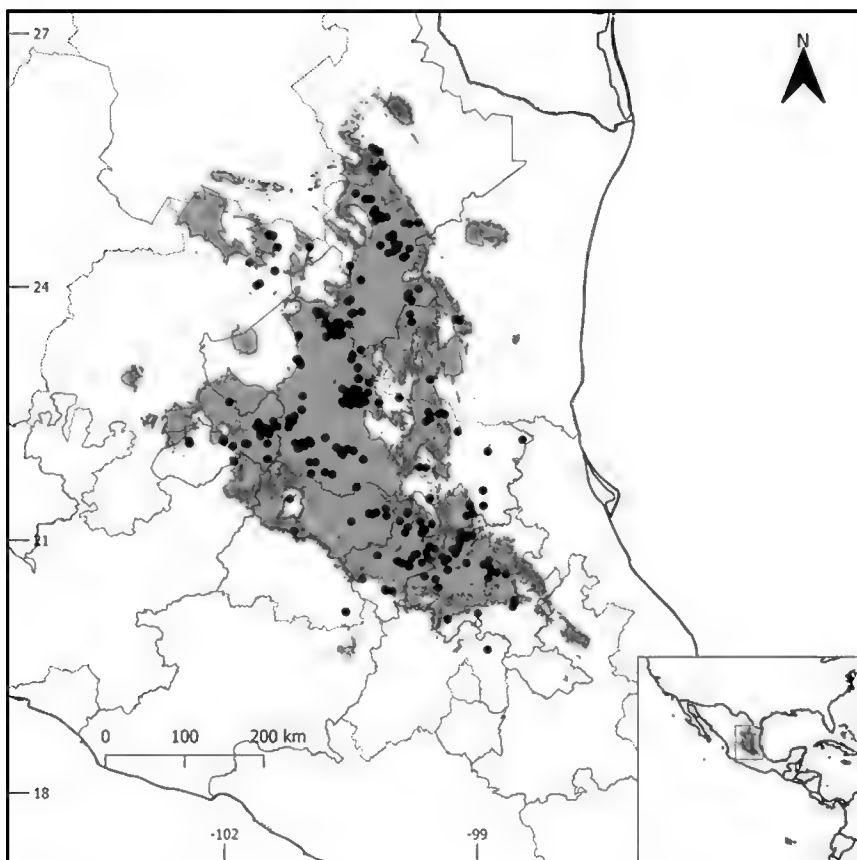
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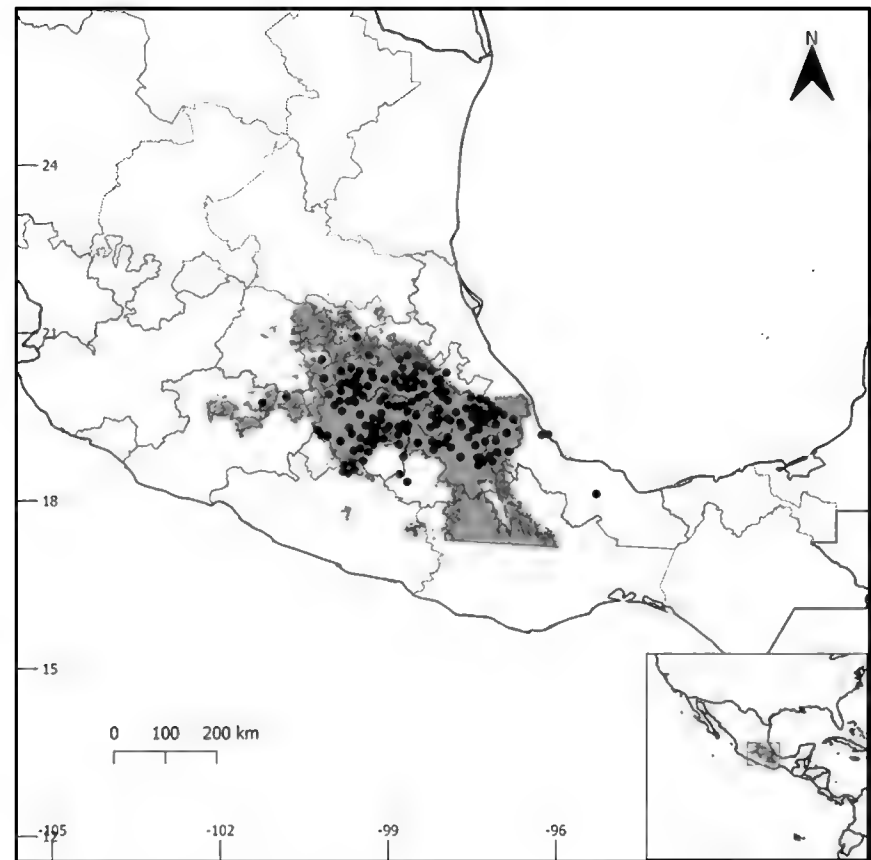
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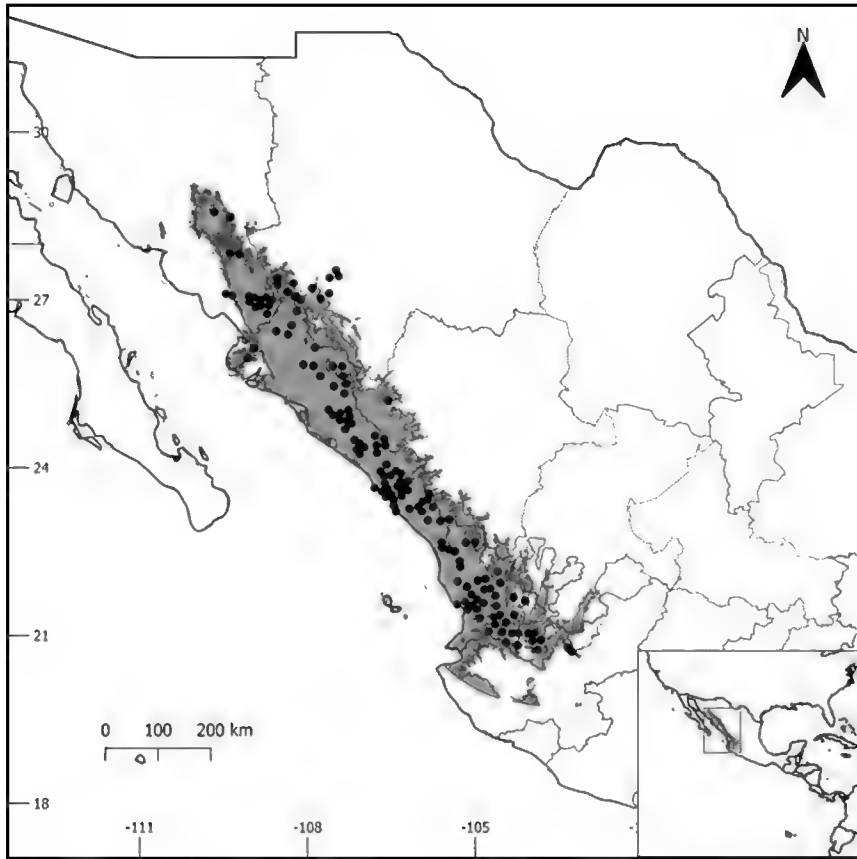
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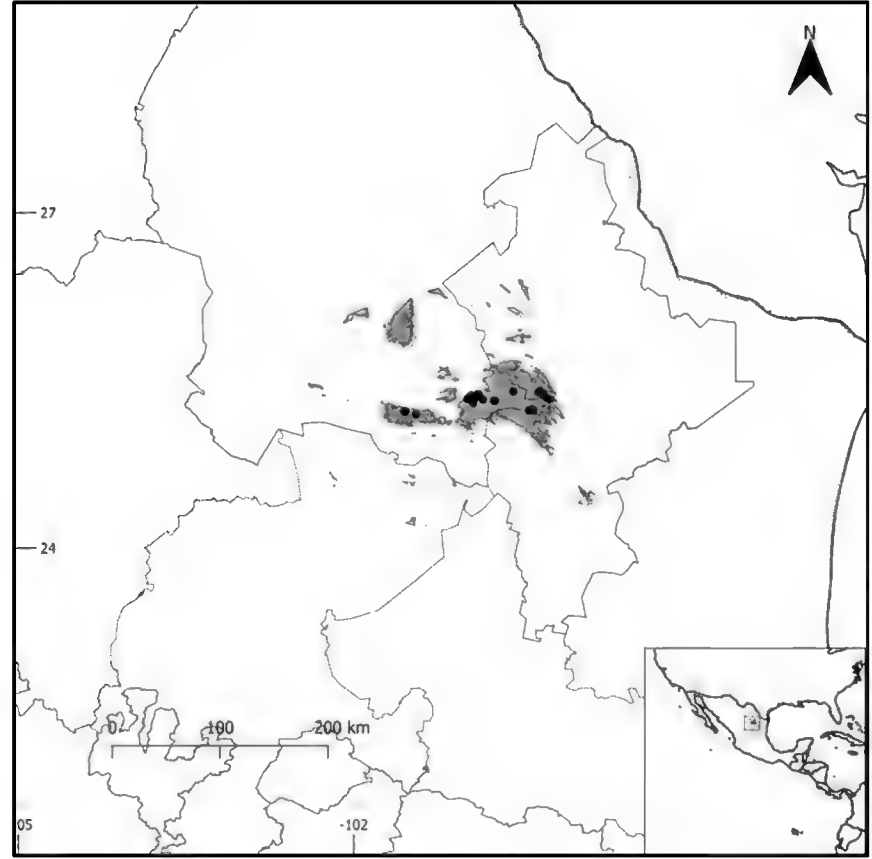
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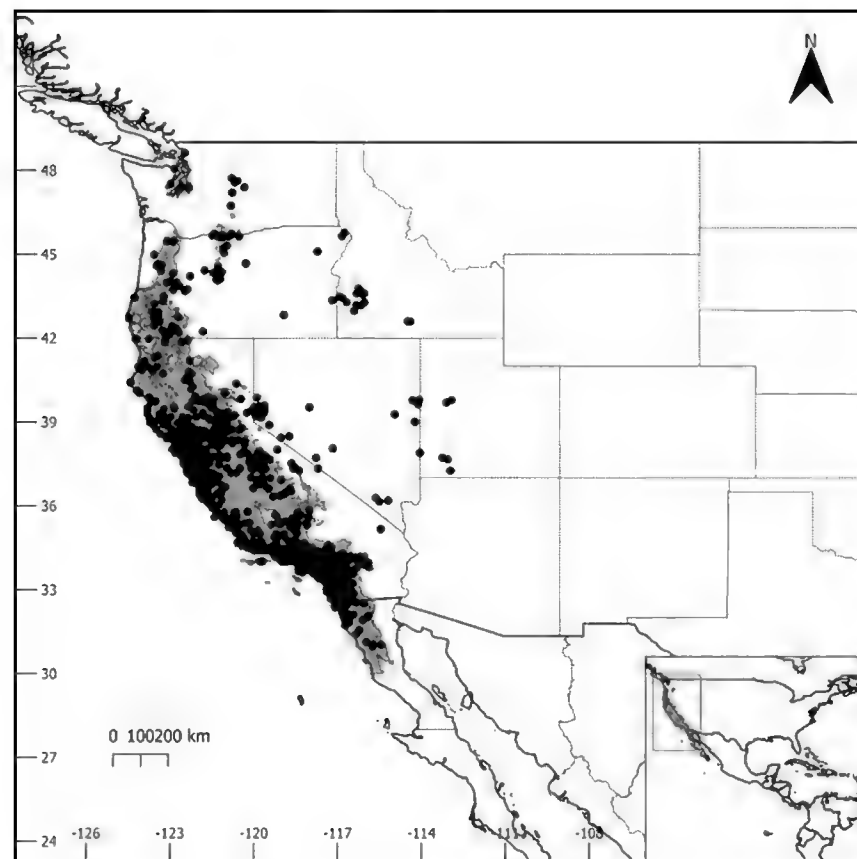
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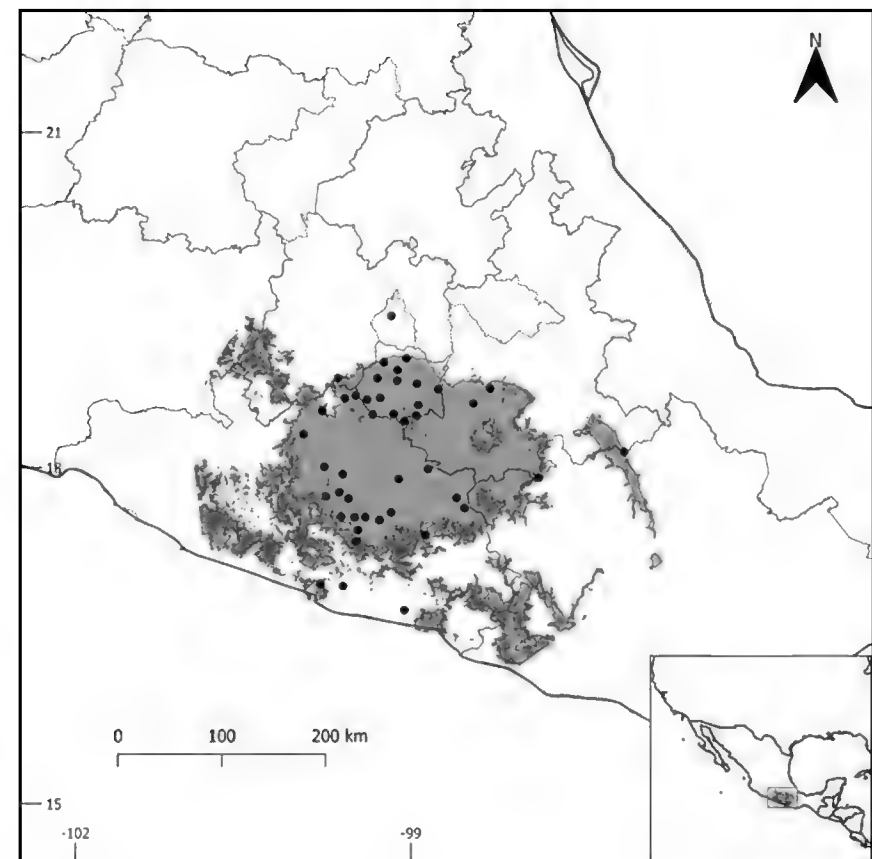
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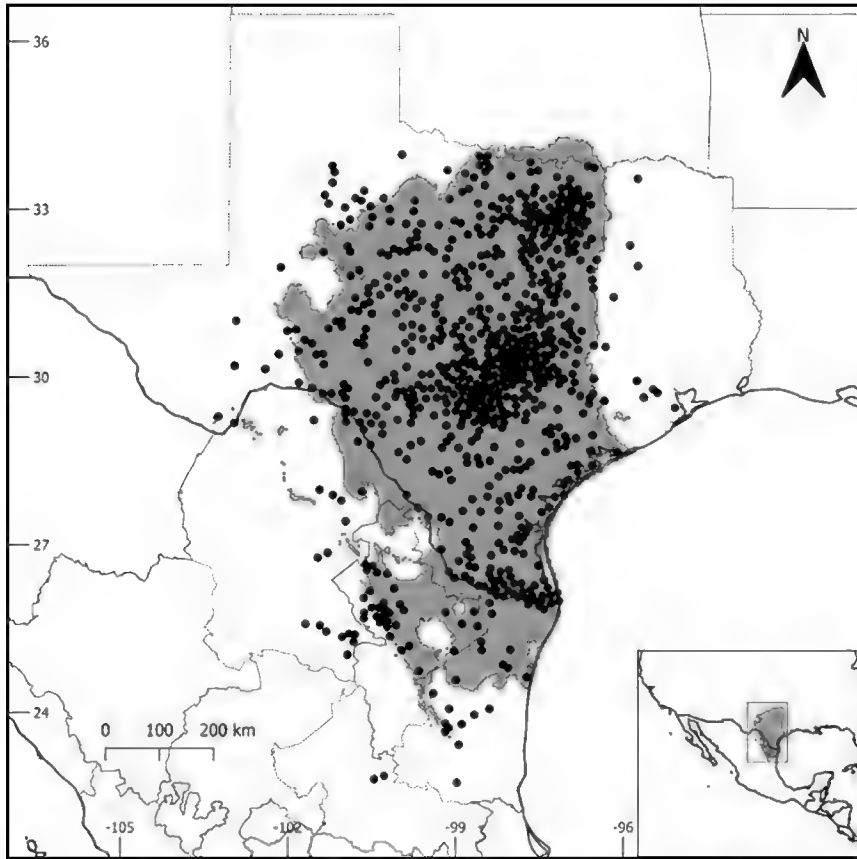
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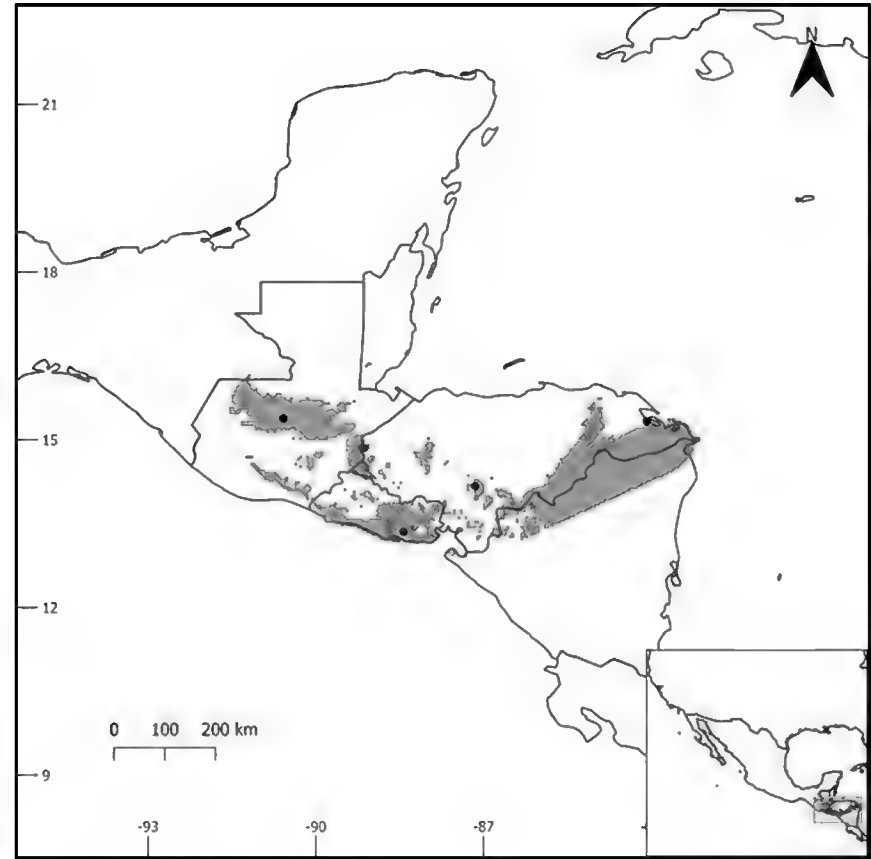
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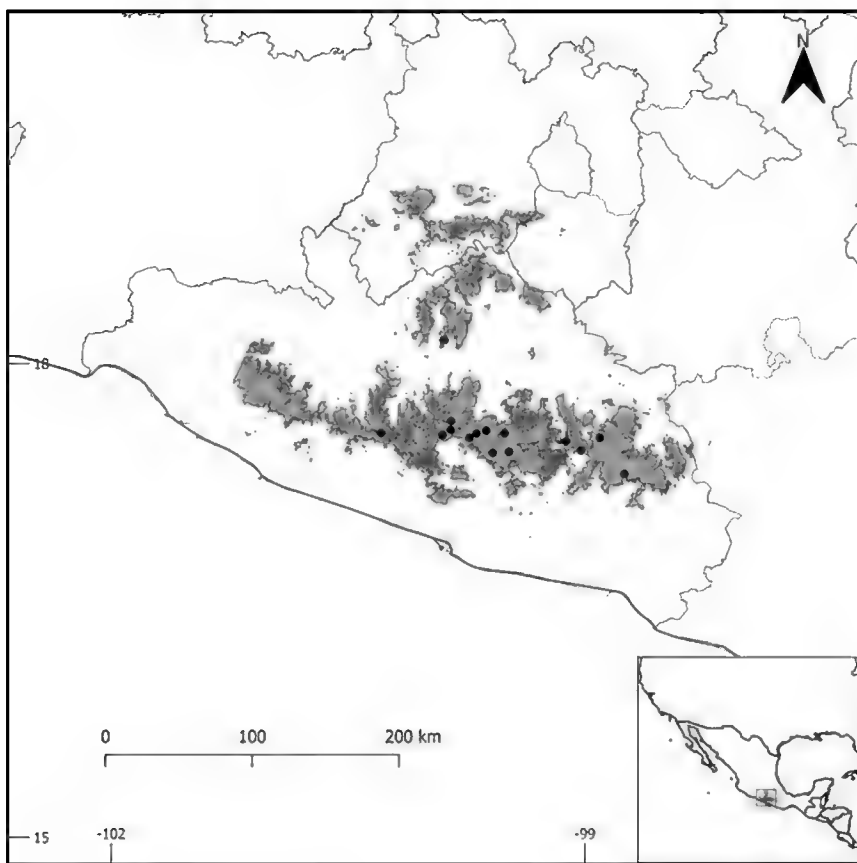
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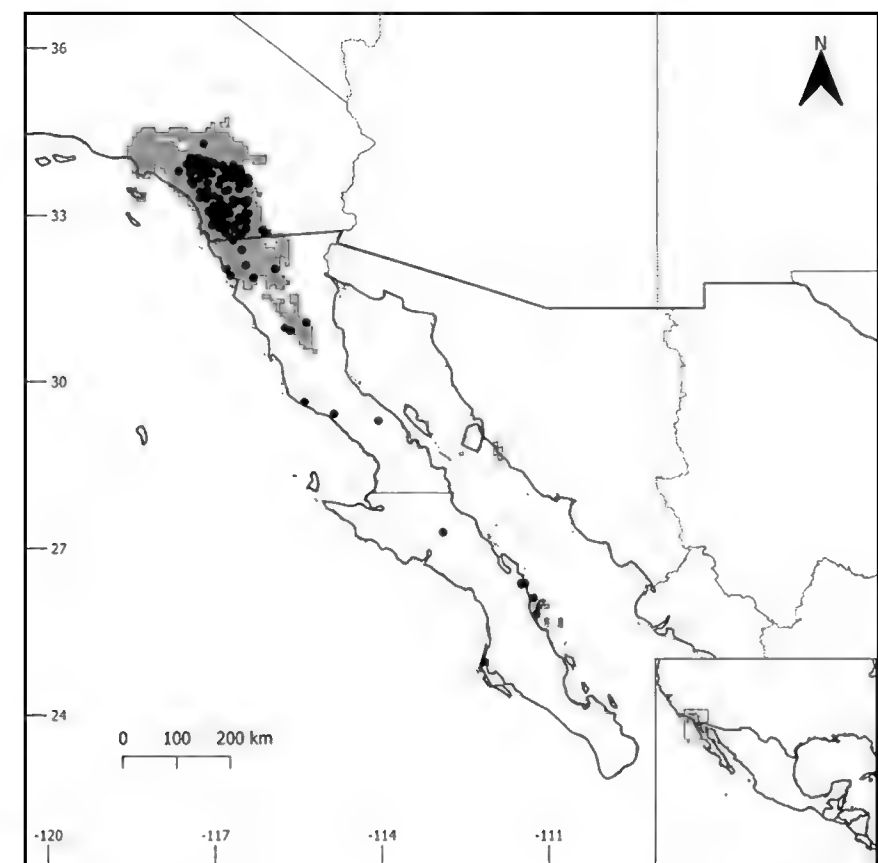
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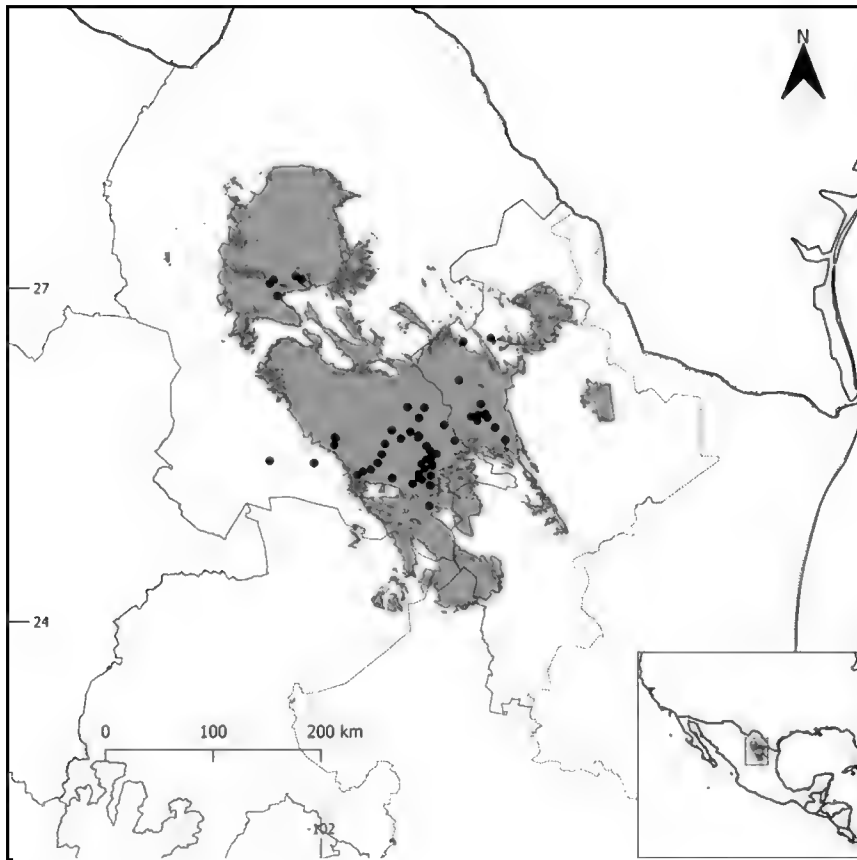
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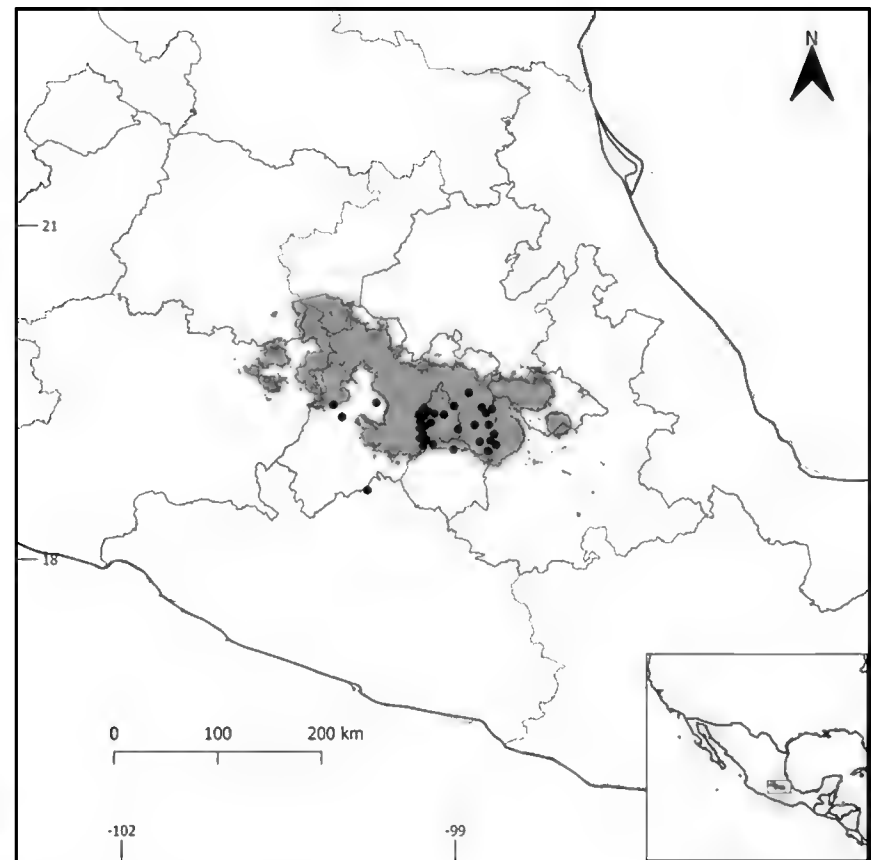
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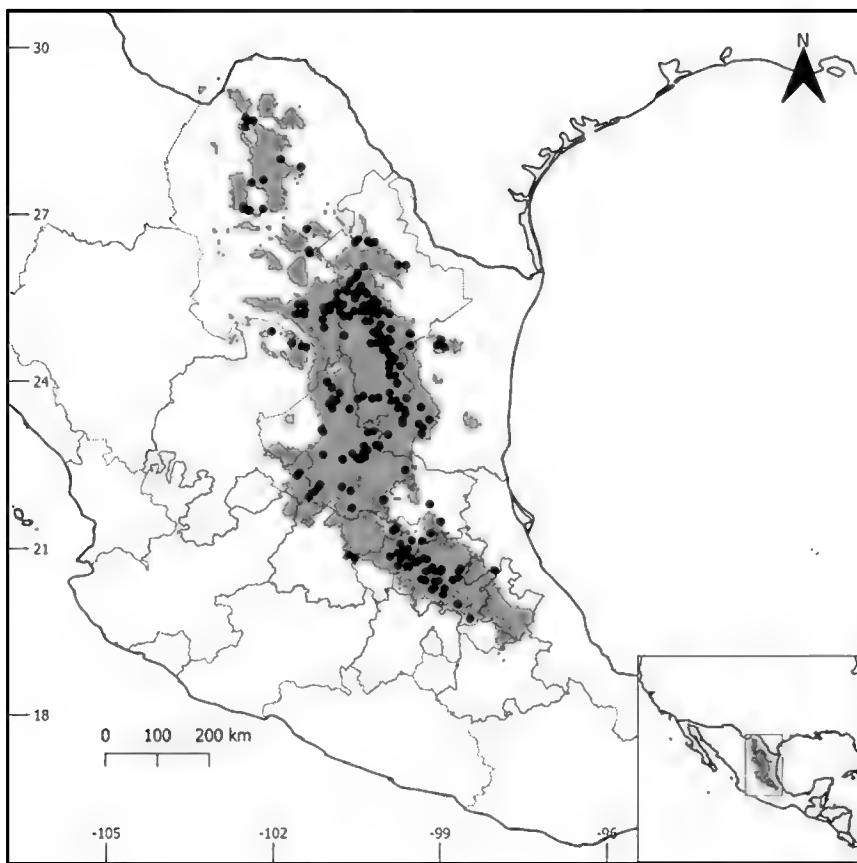
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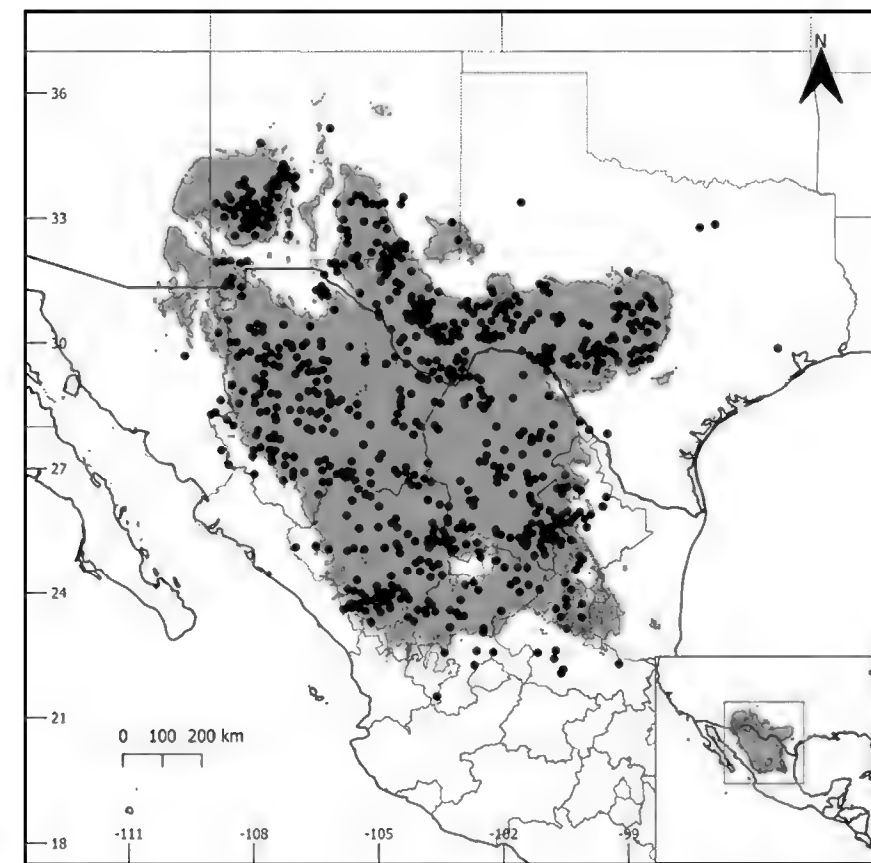
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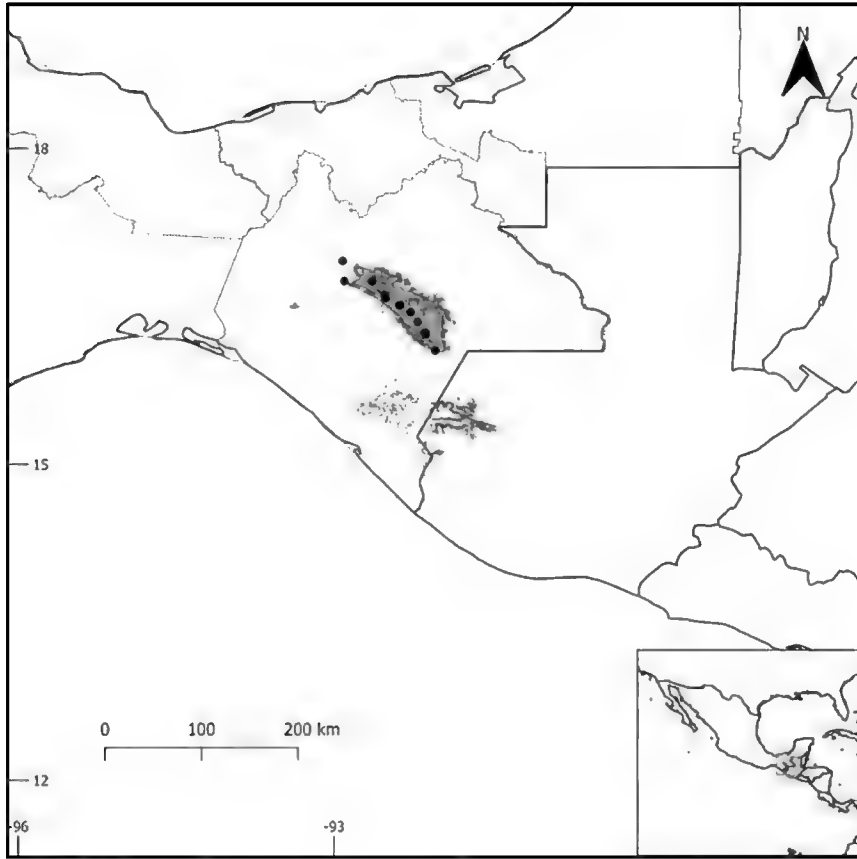
Sceloporus palaciosi



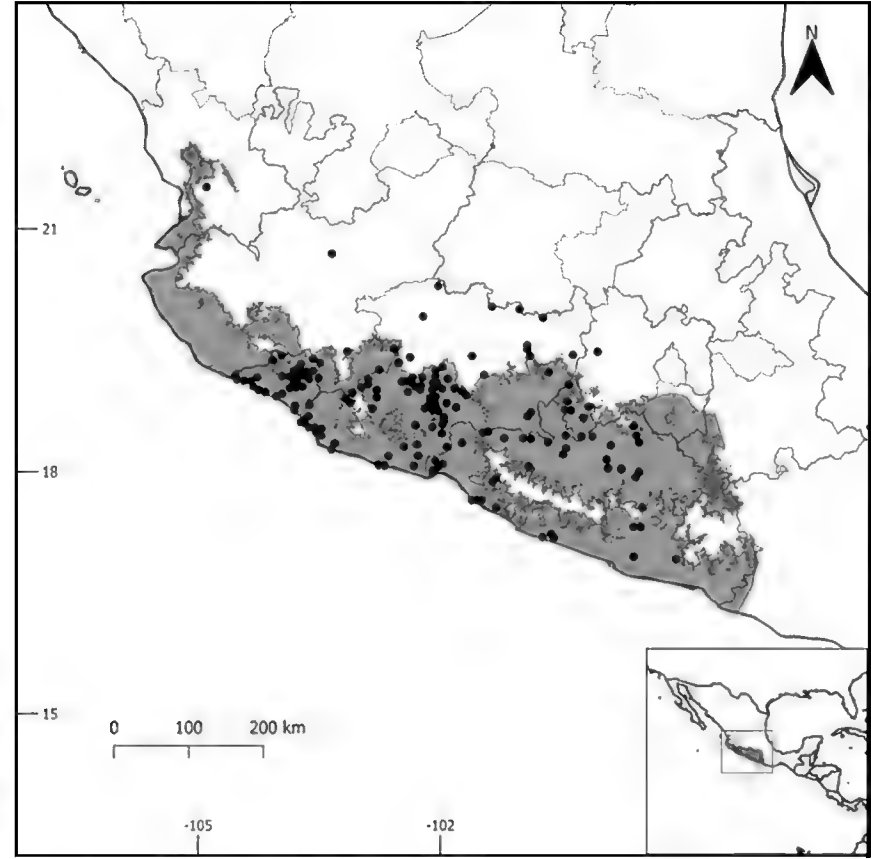
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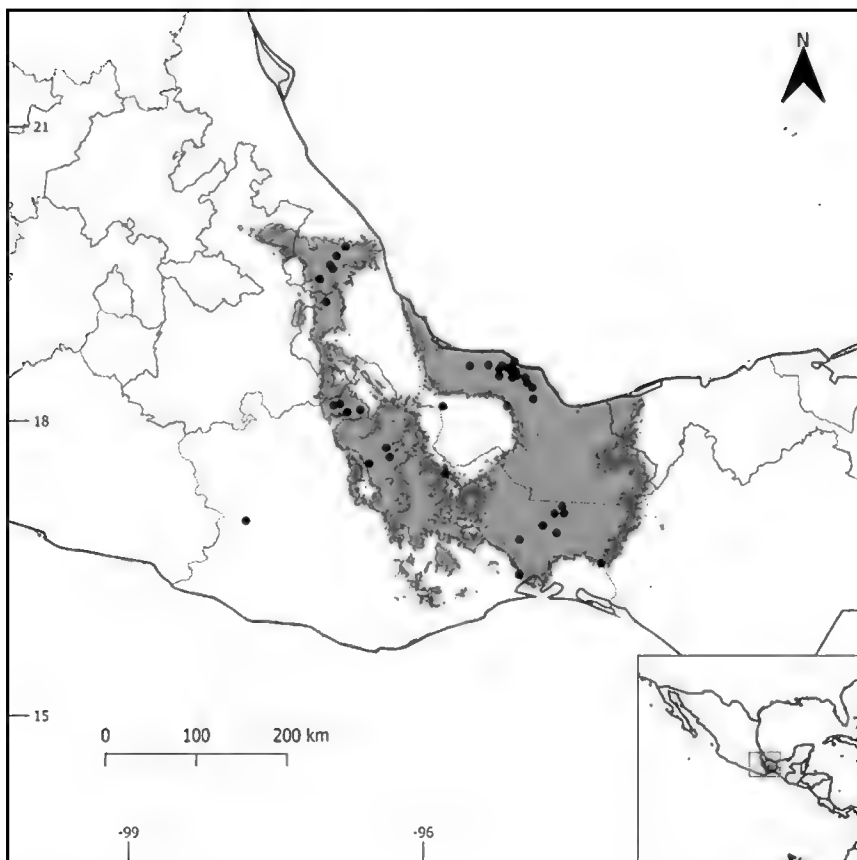
Sceloporus poinsettii



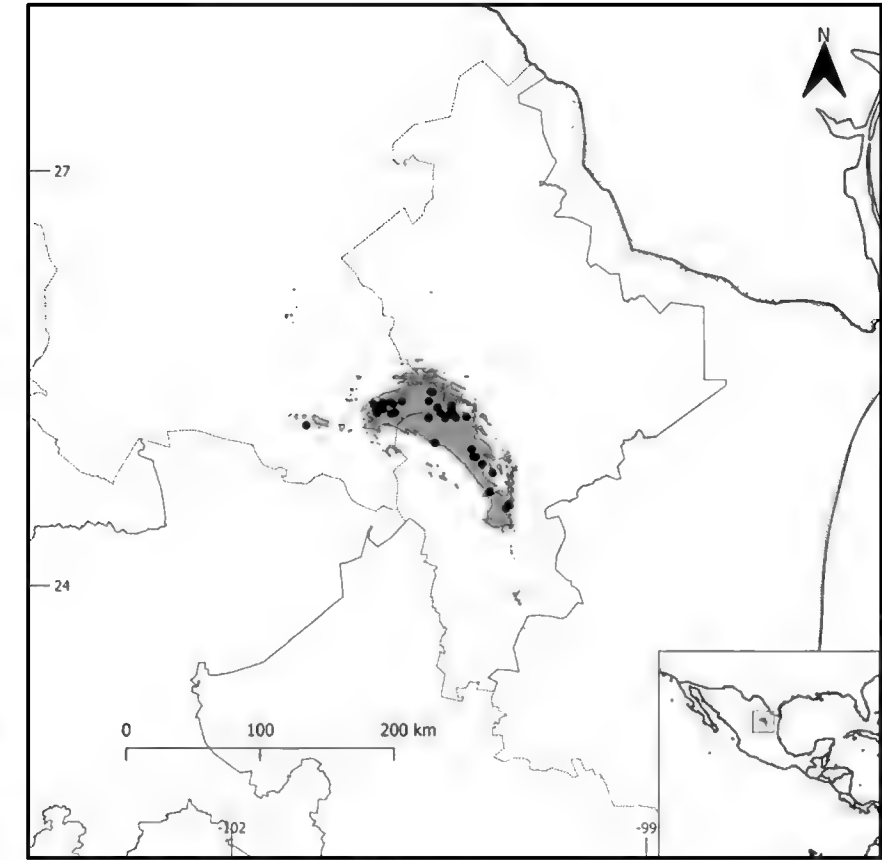
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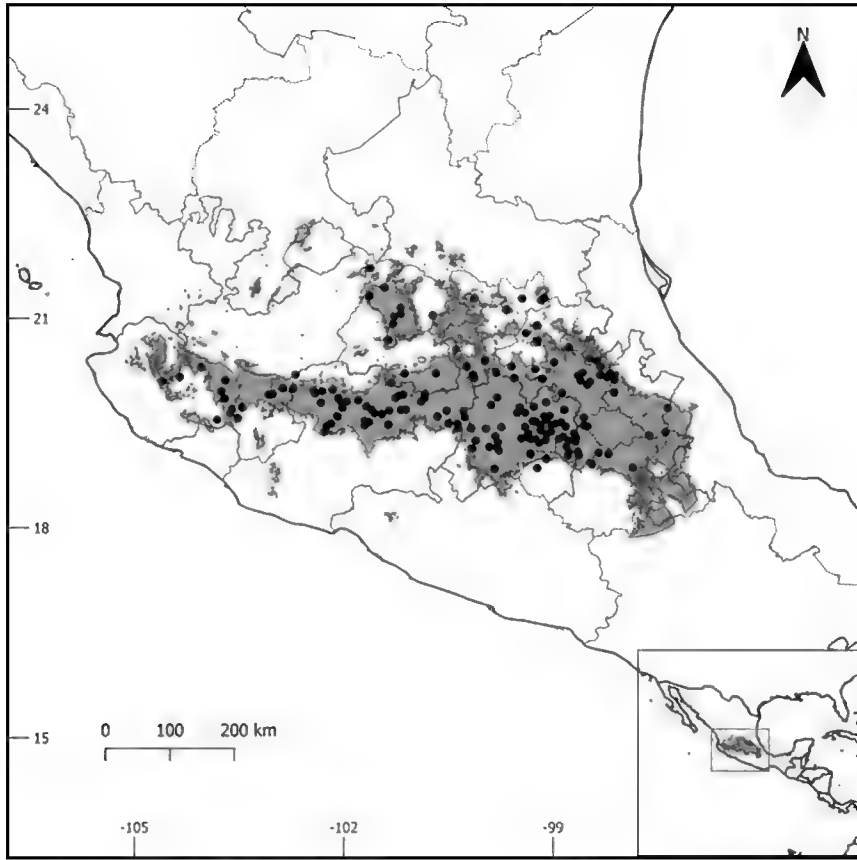
Sceloporus pyrocephalus



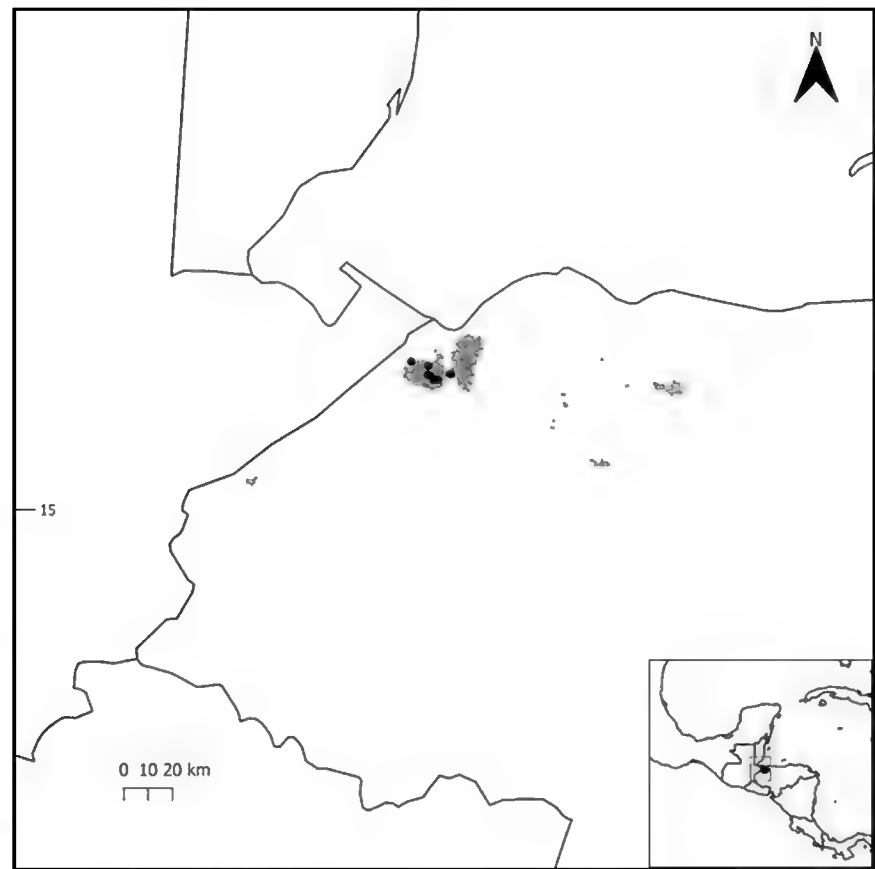
Sceloporus salvini



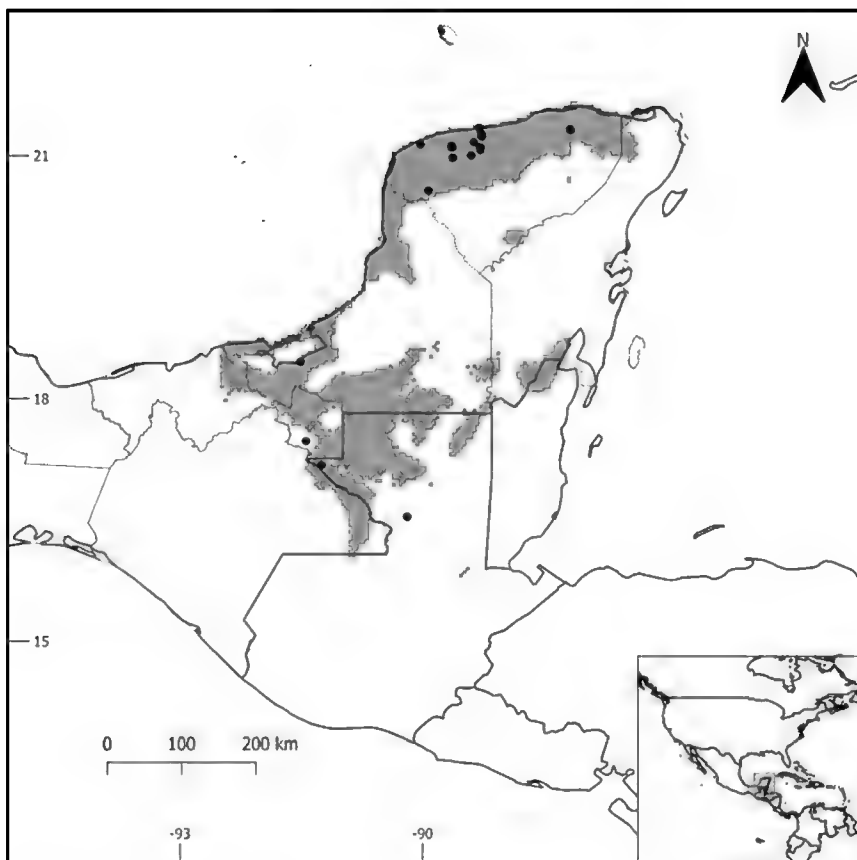
Sceloporus samcolemani



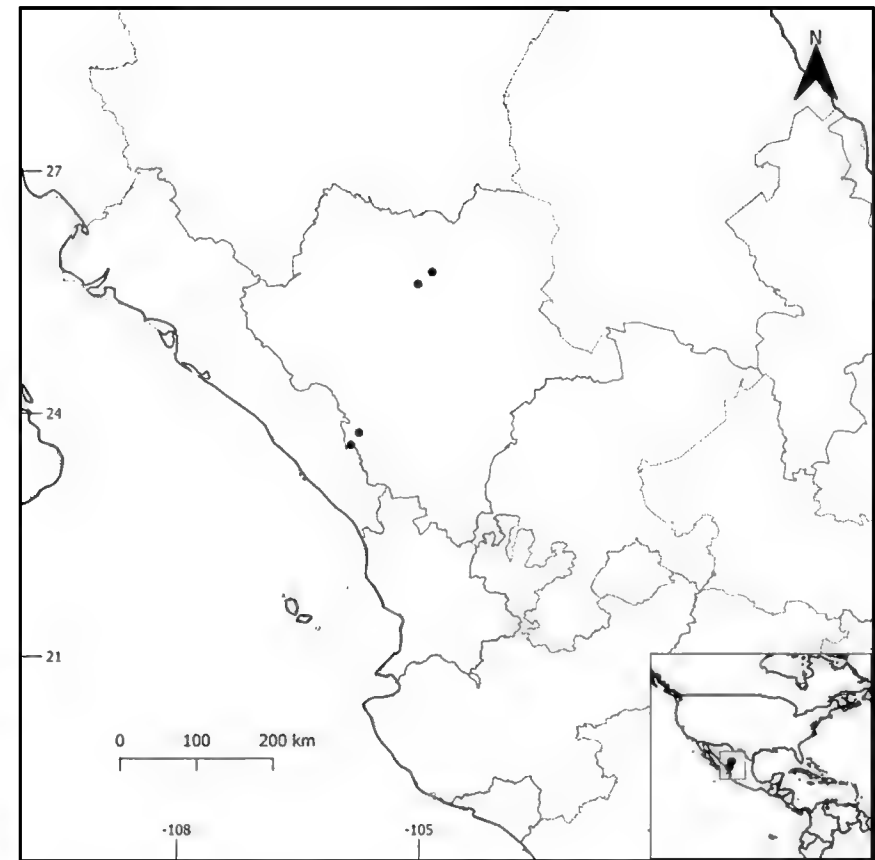
Sceloporus scalaris



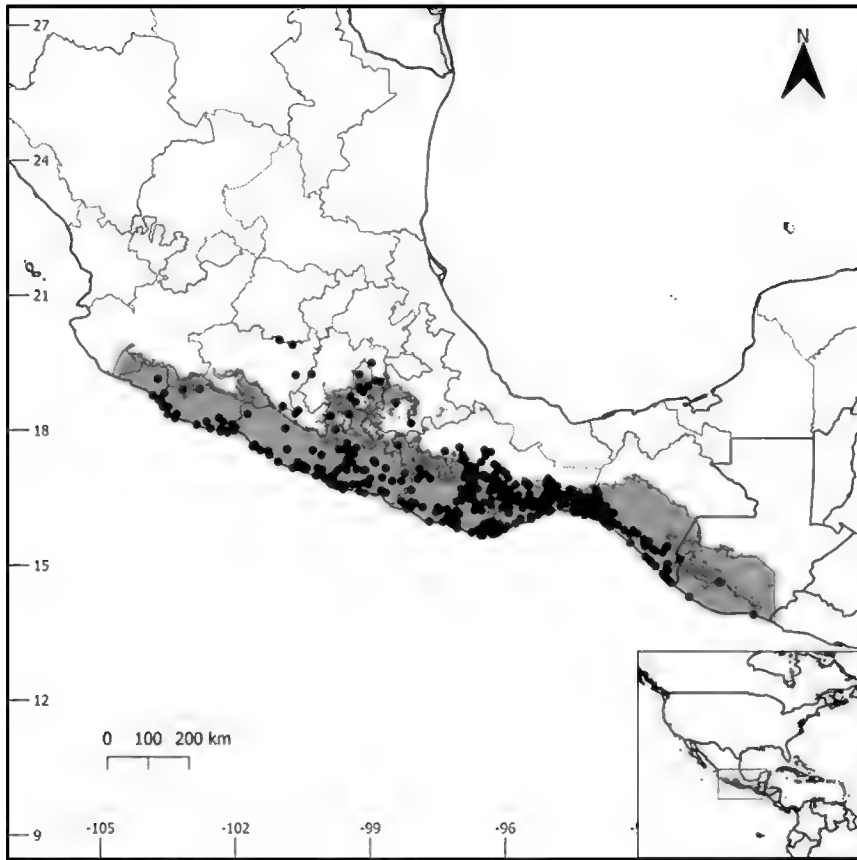
Sceloporus schmidtii



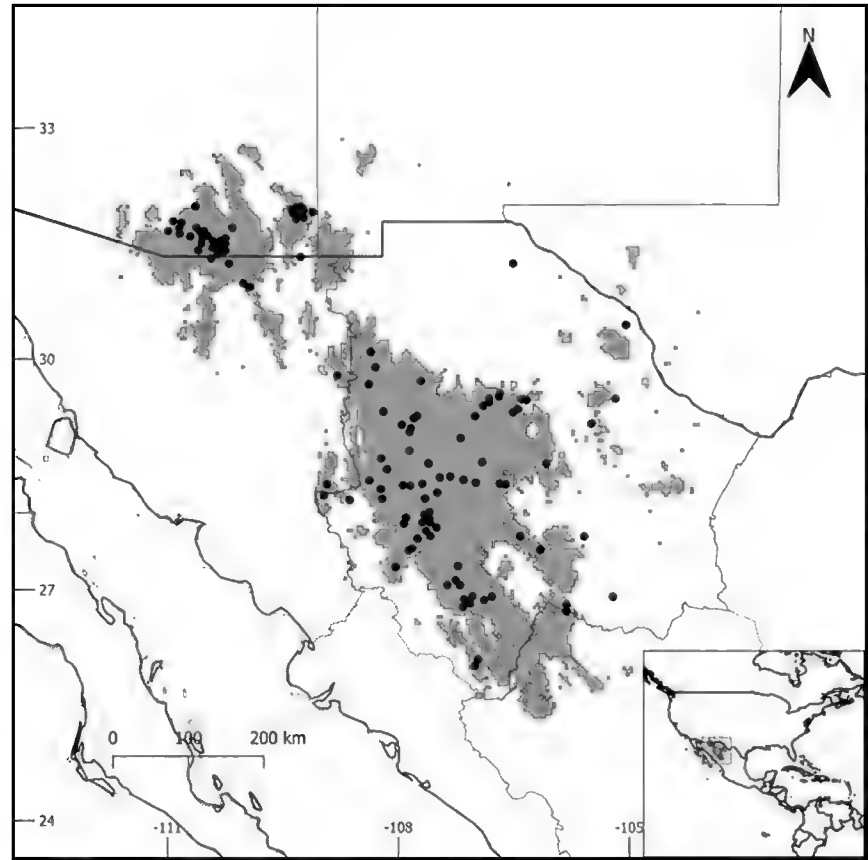
Sceloporus serrifer



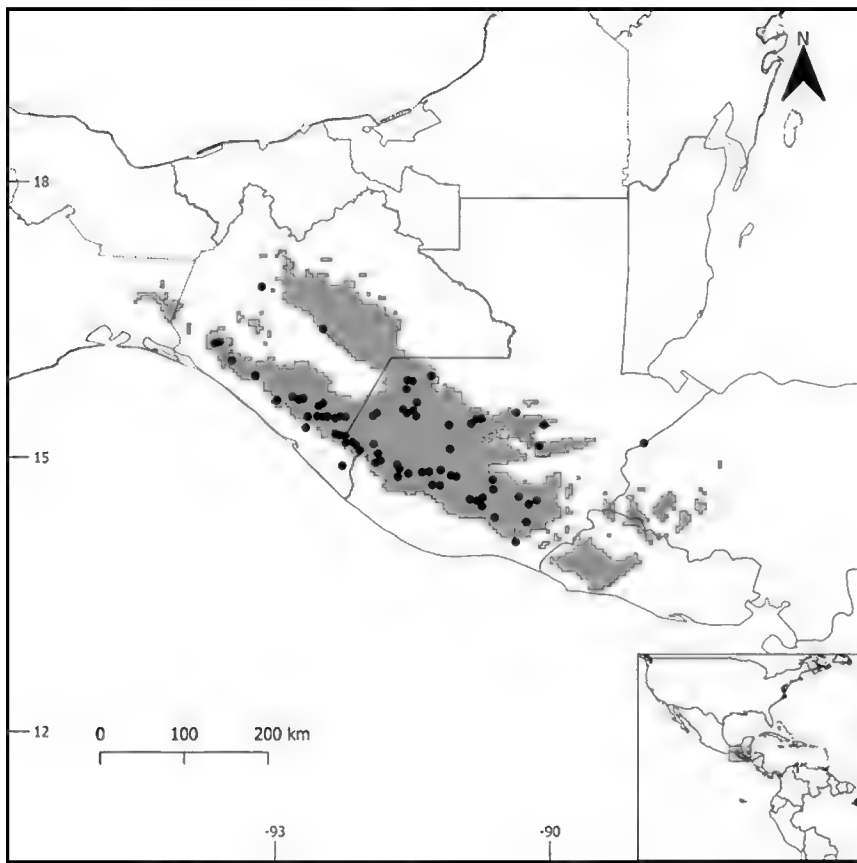
Sceloporus shannonorum



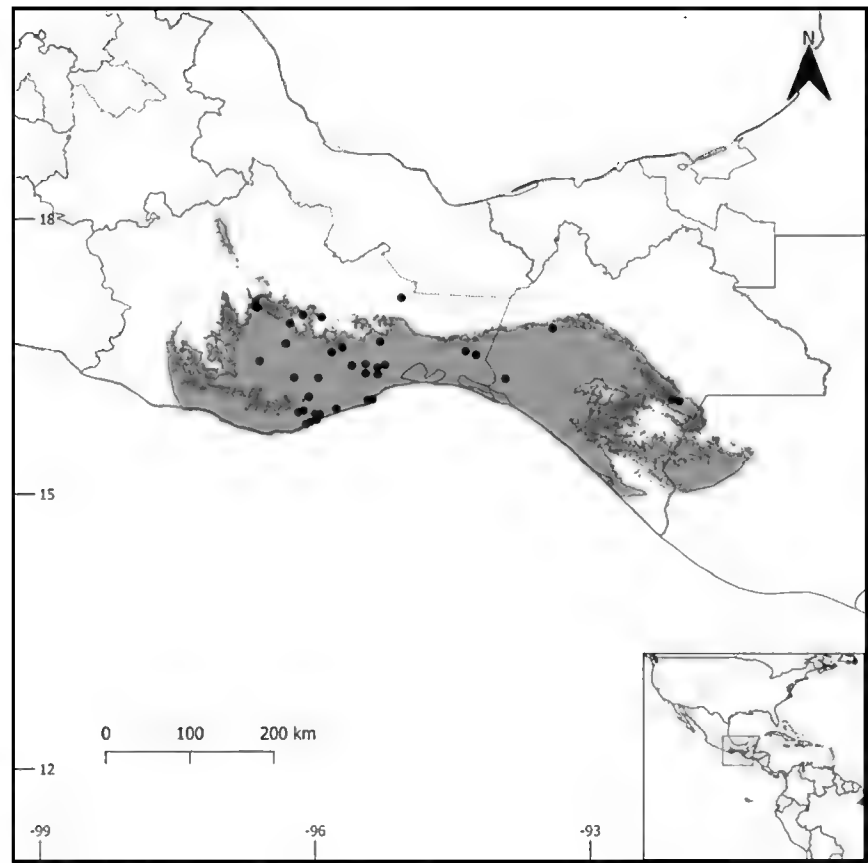
Sceloporus siniferus



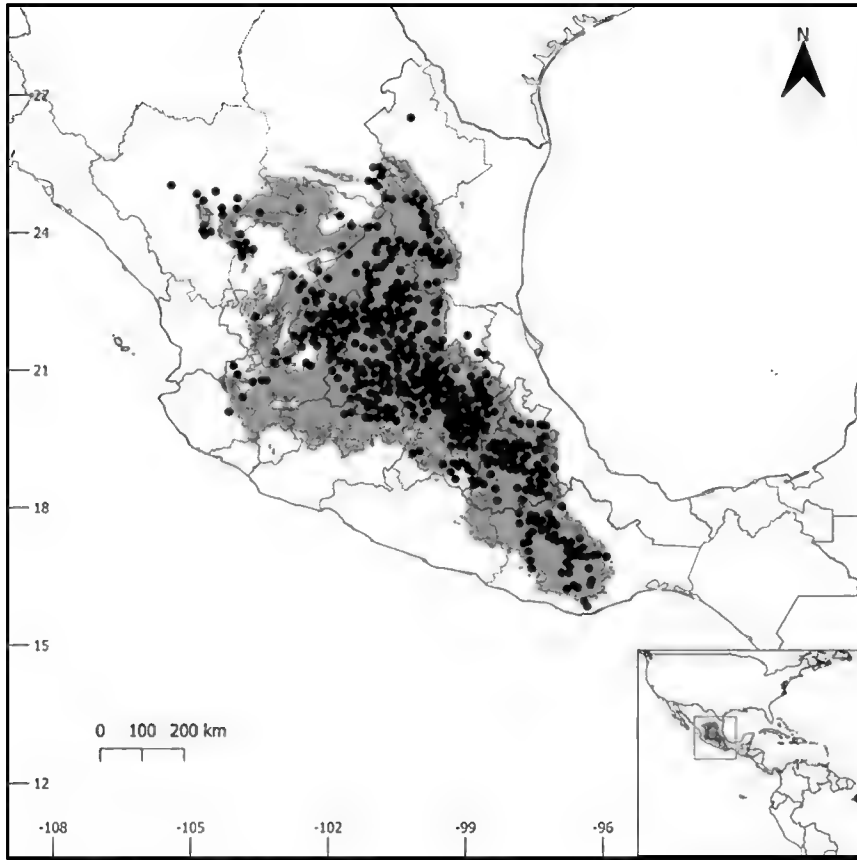
Sceloporus slewini



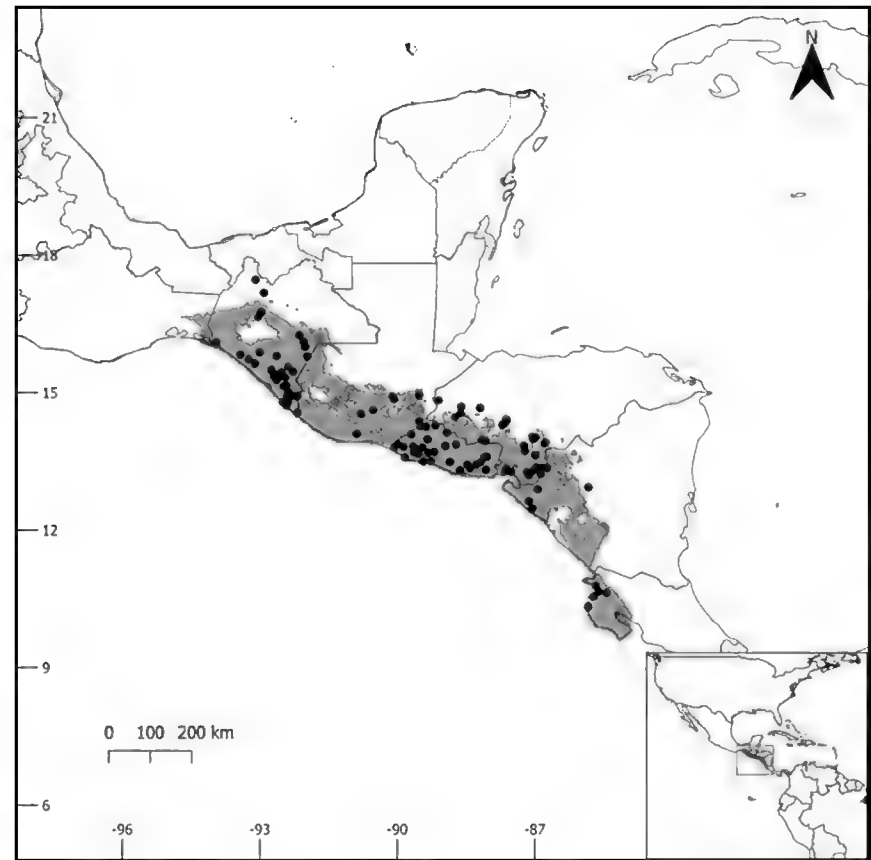
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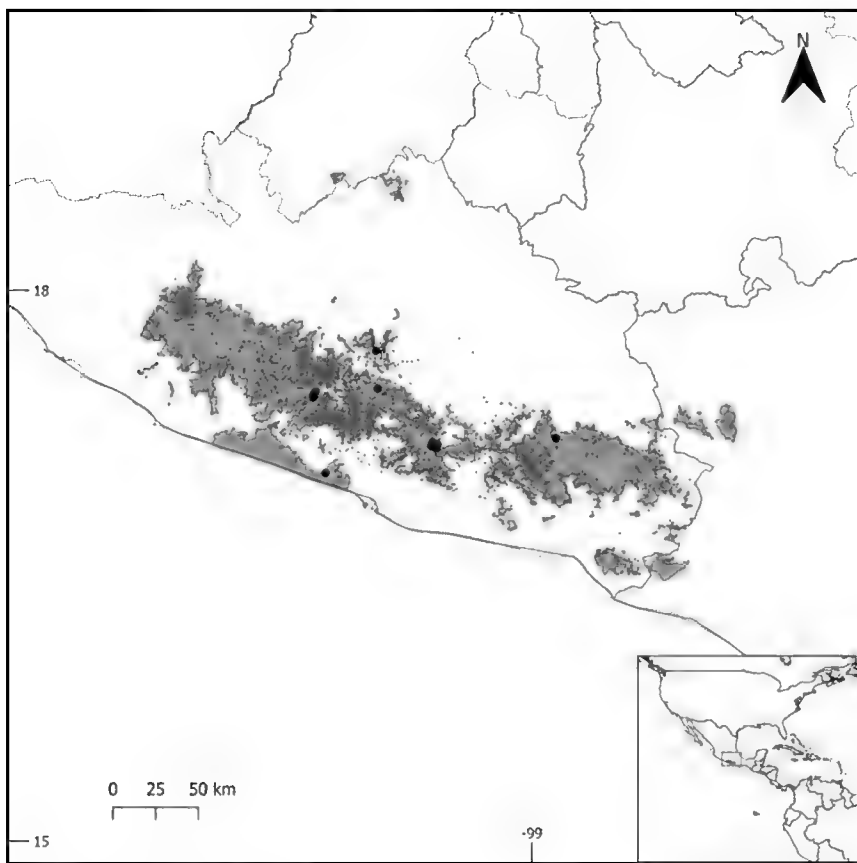
Sceloporus smithi



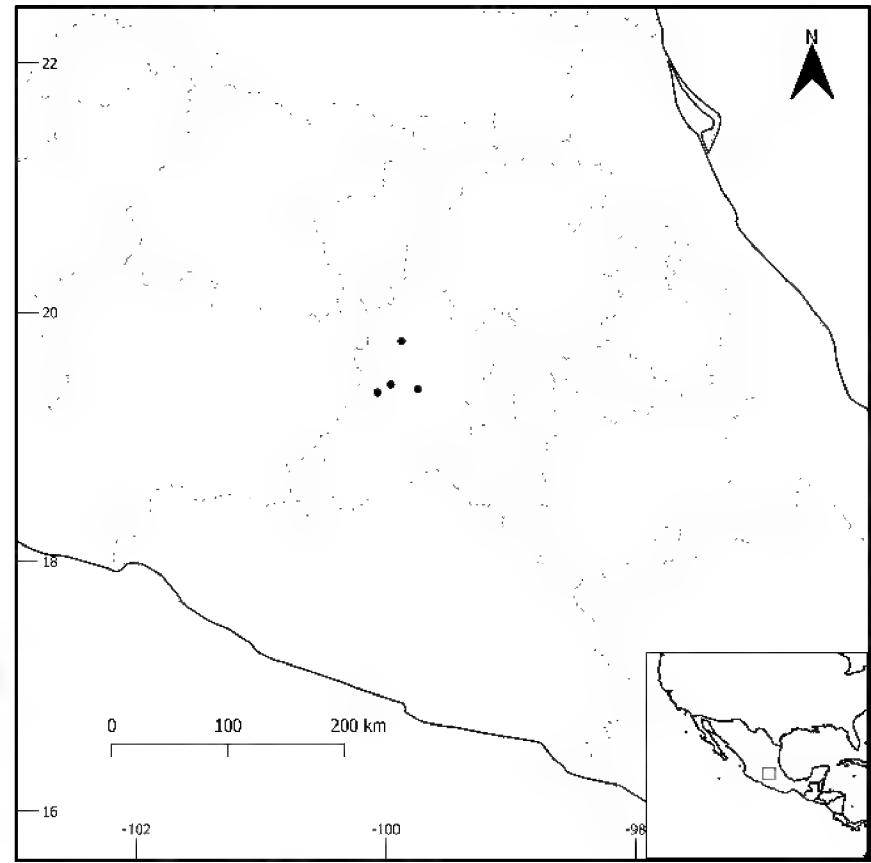
Sceloporus spinosus



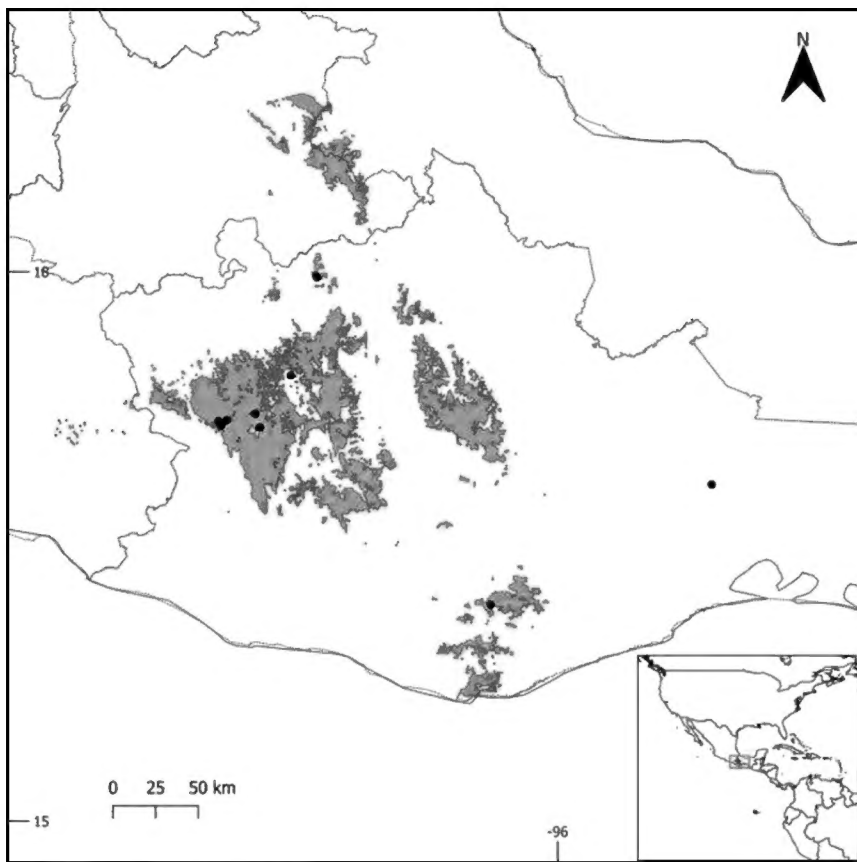
Sceloporus squamosus



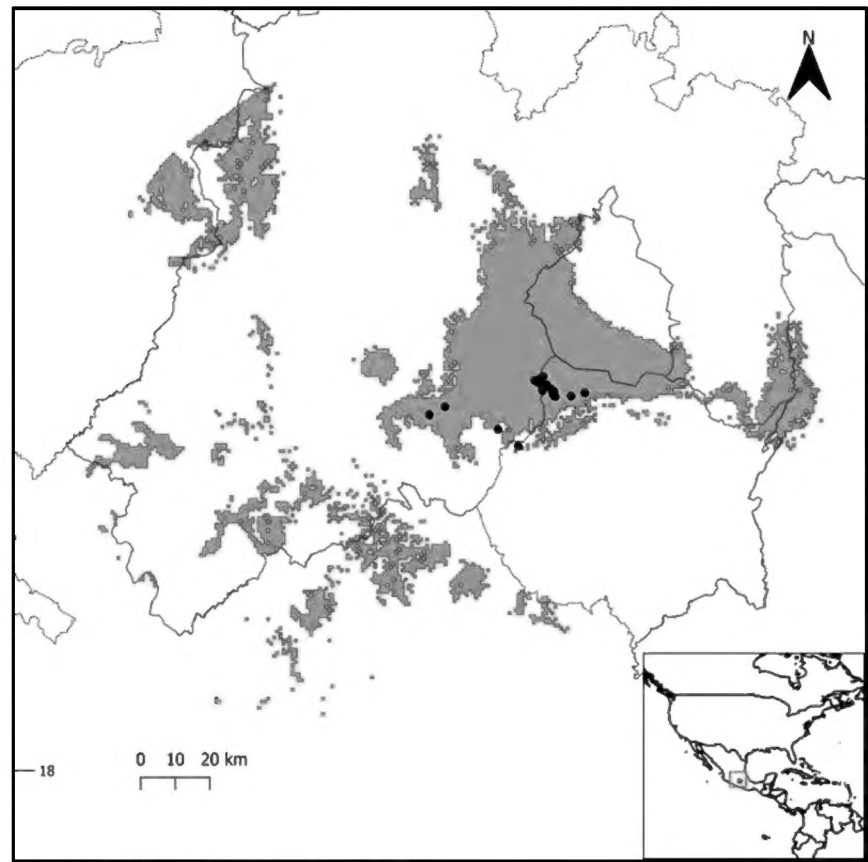
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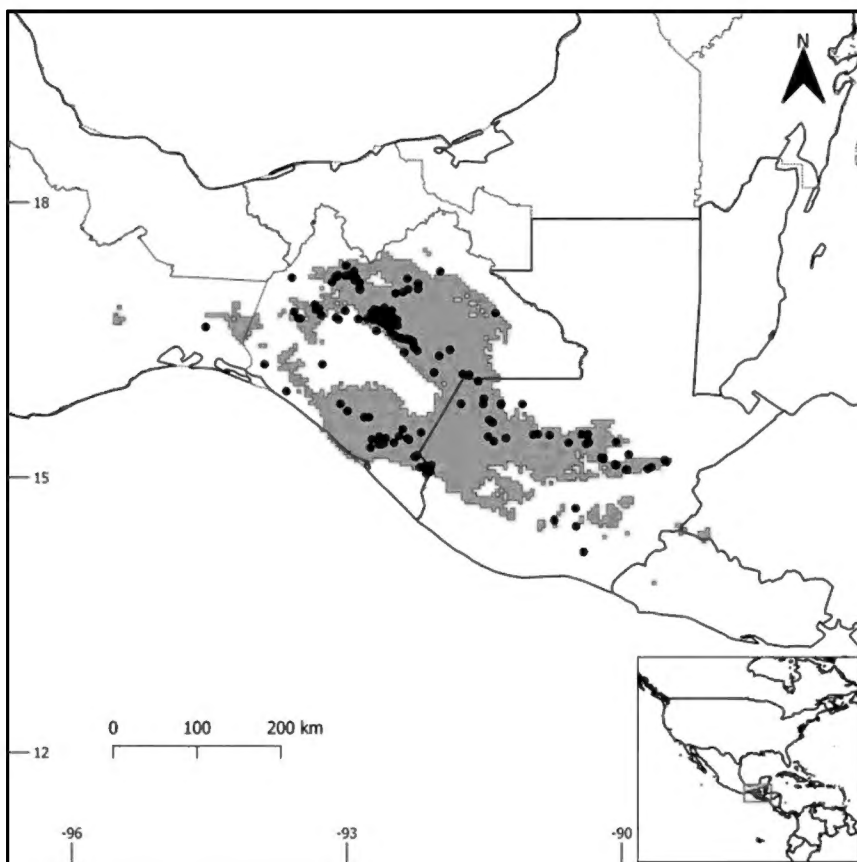
Sceloporus subniger



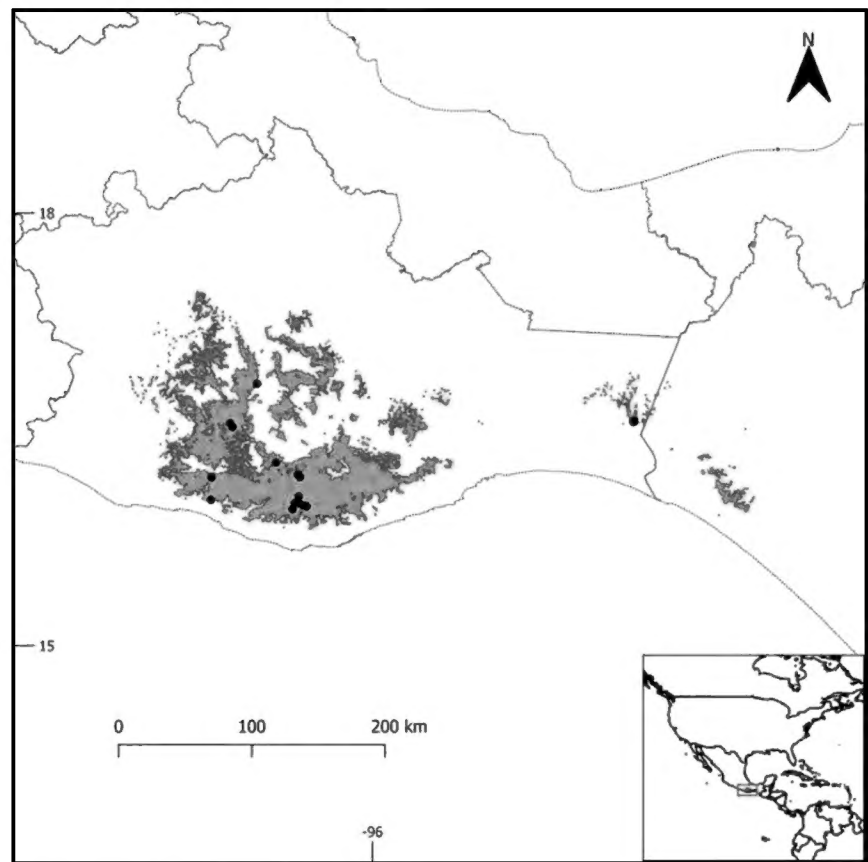
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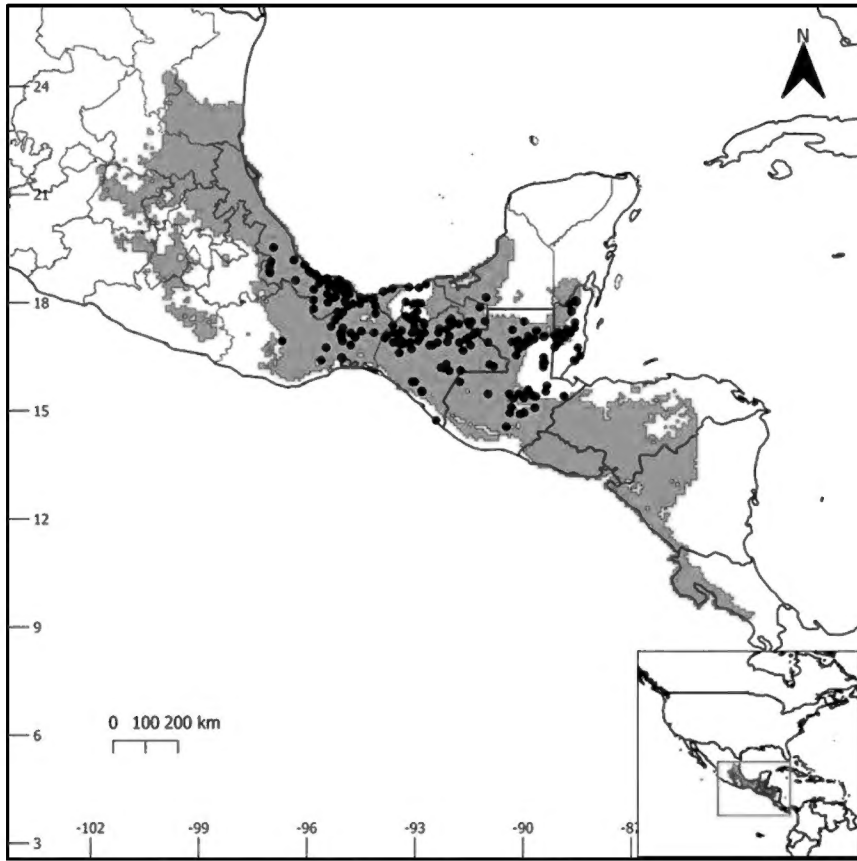
Sceloporus sugillatus



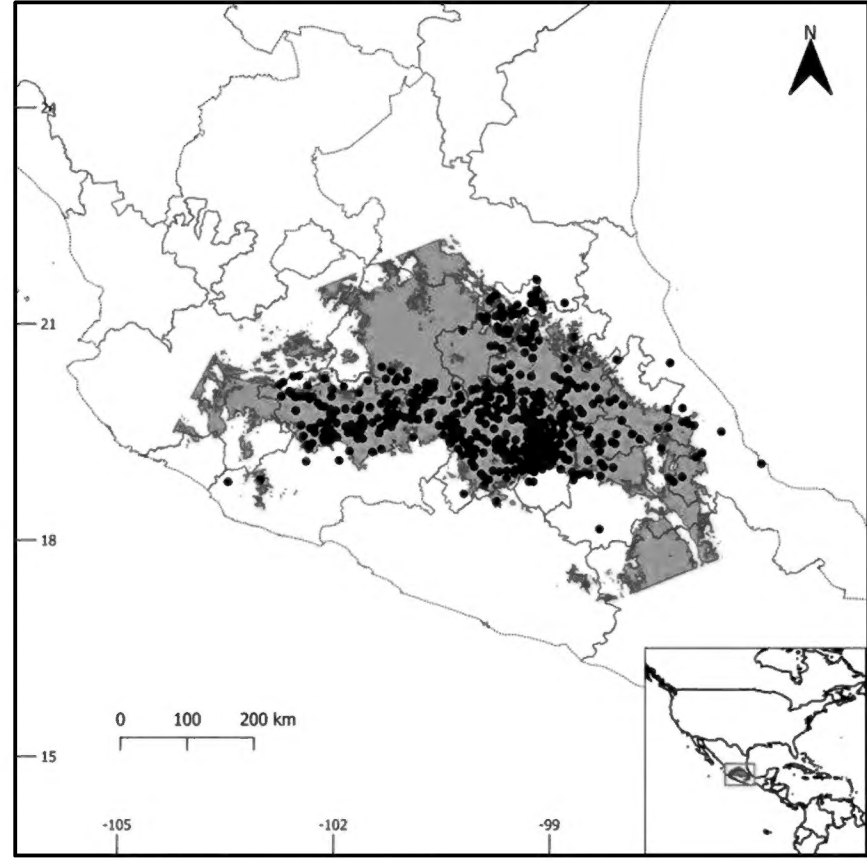
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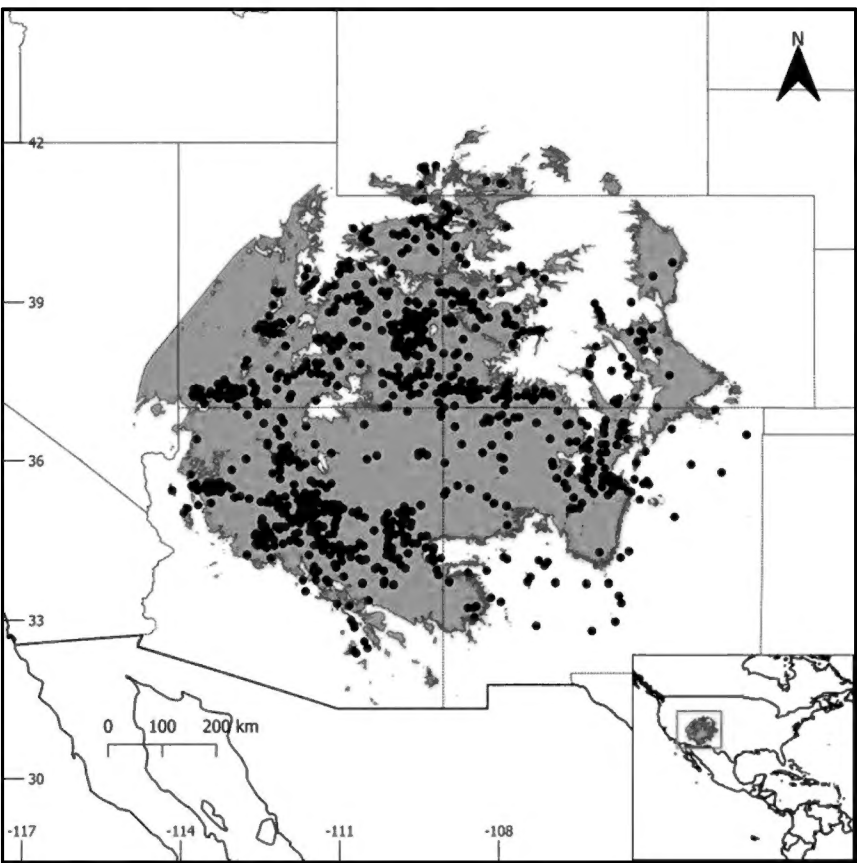
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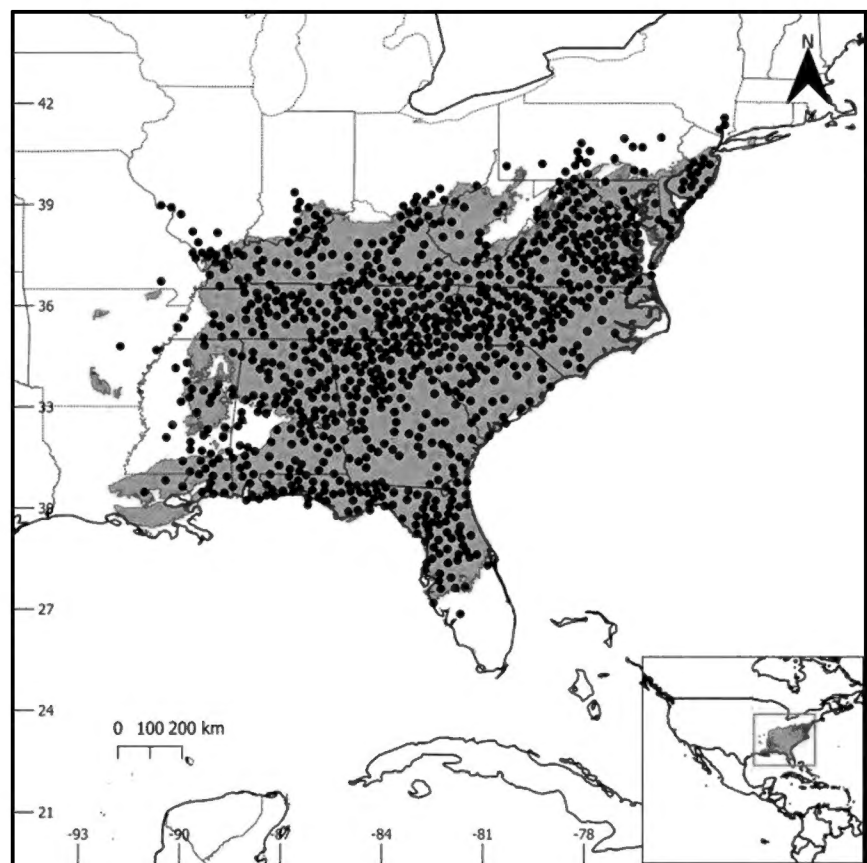
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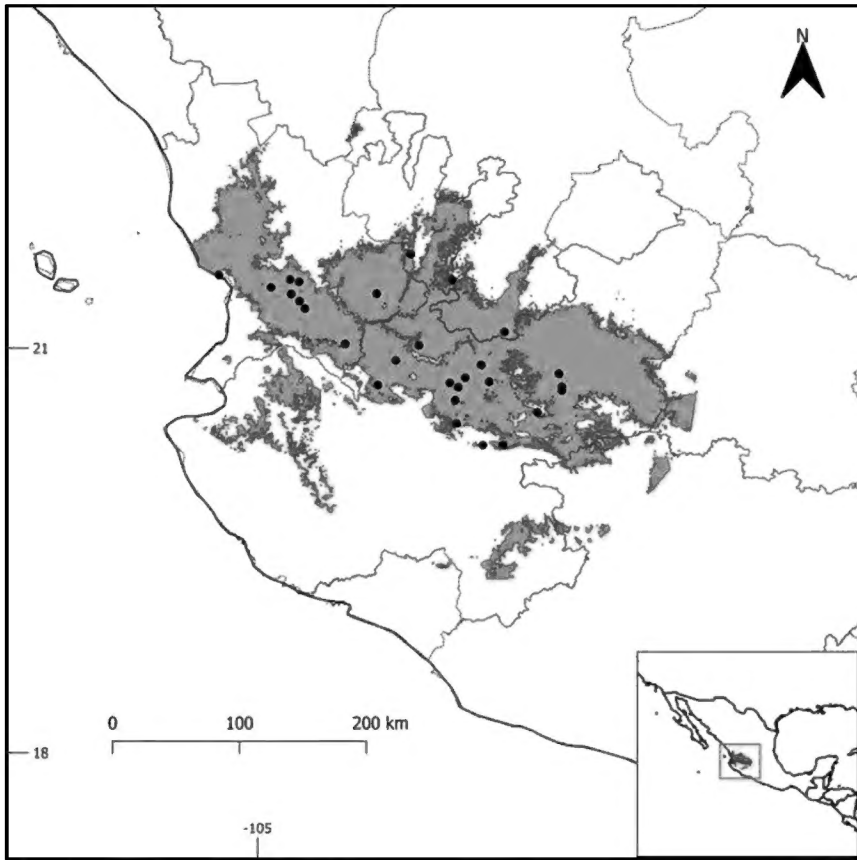
Sceloporus torquatus



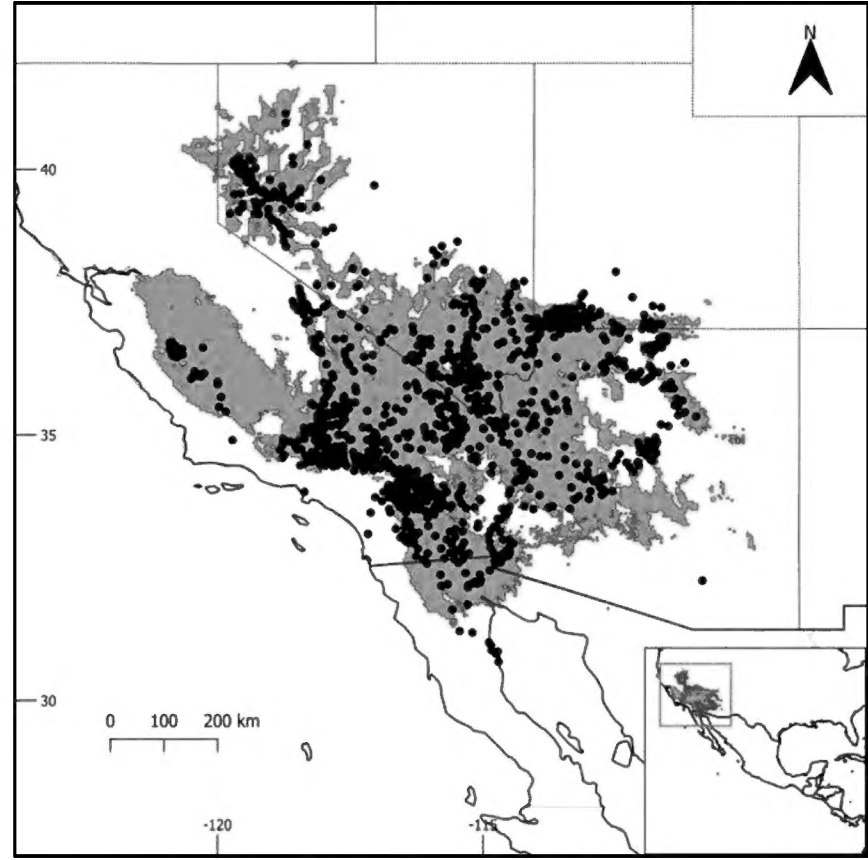
Sceloporus tristichus



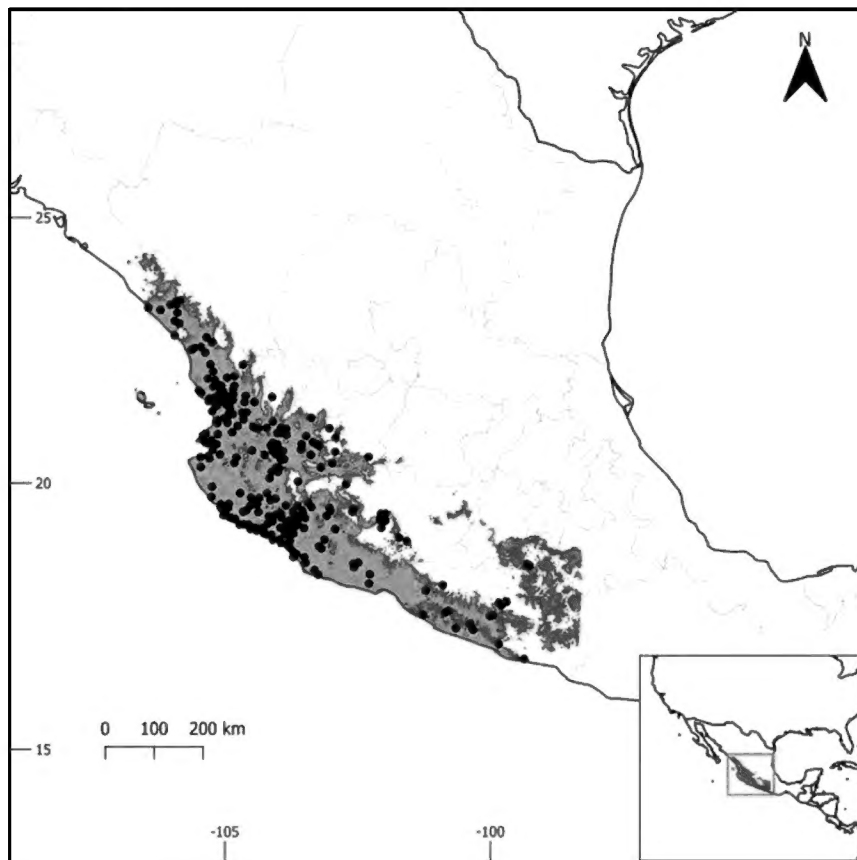
Sceloporus undulatus



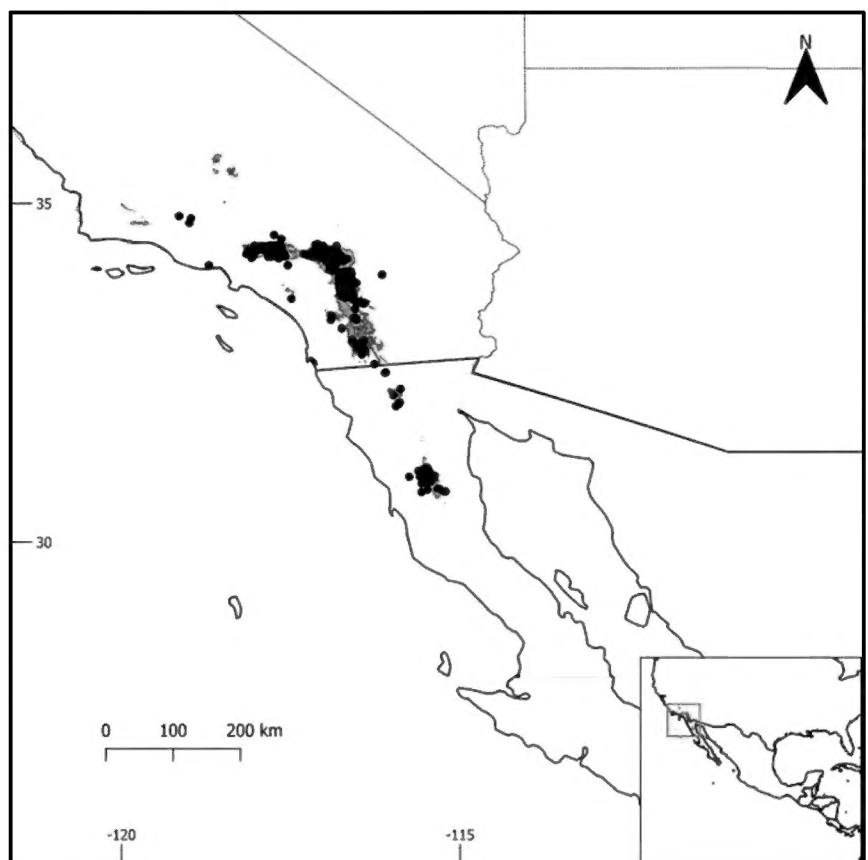
Sceloporus unicanthalis



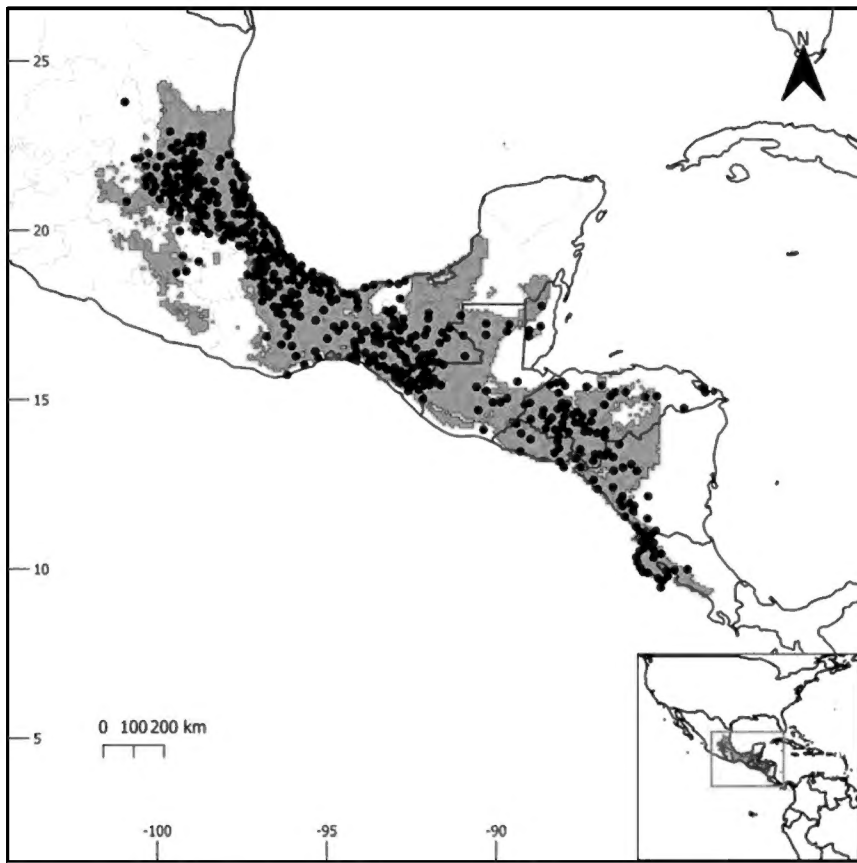
Sceloporus uniformis



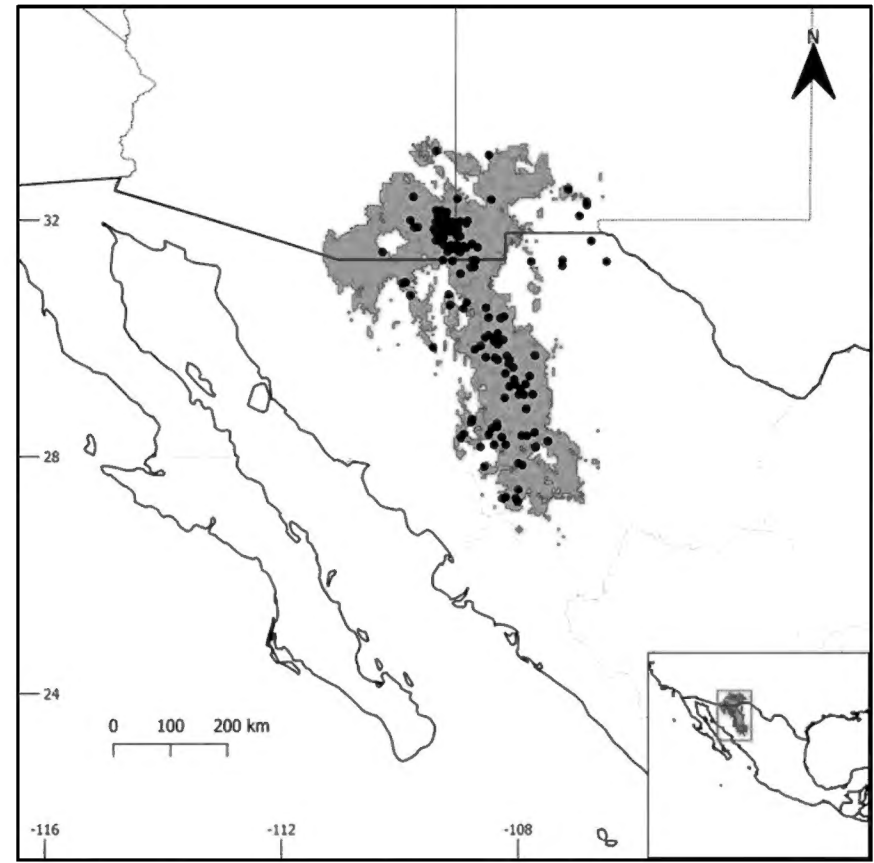
Sceloporus utiformis



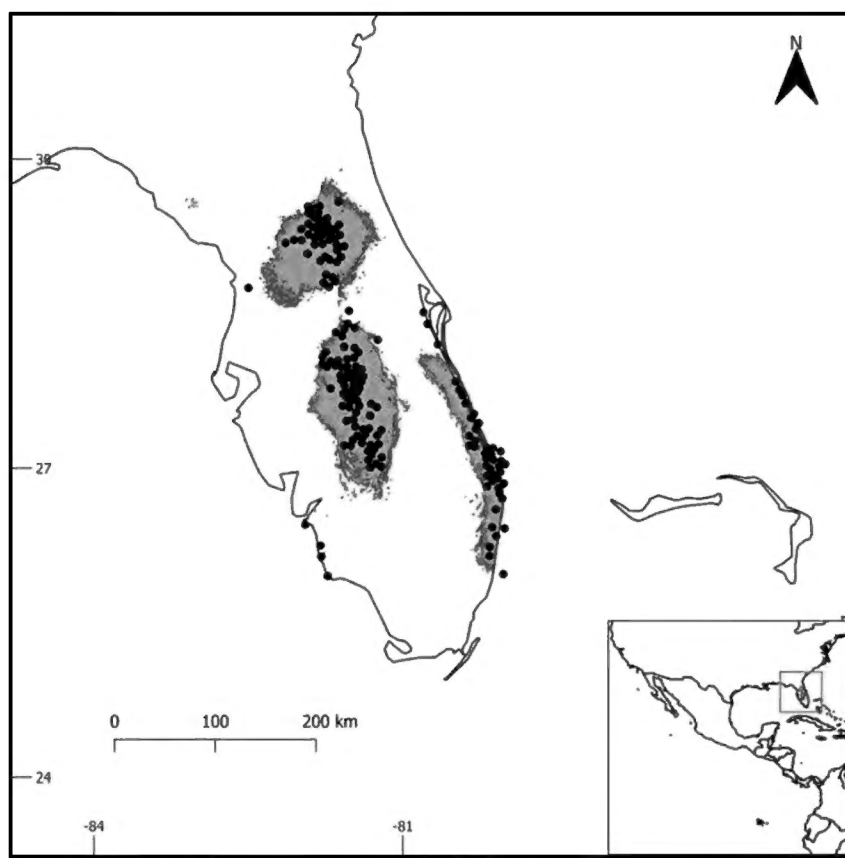
Sceloporus vandenburgianus



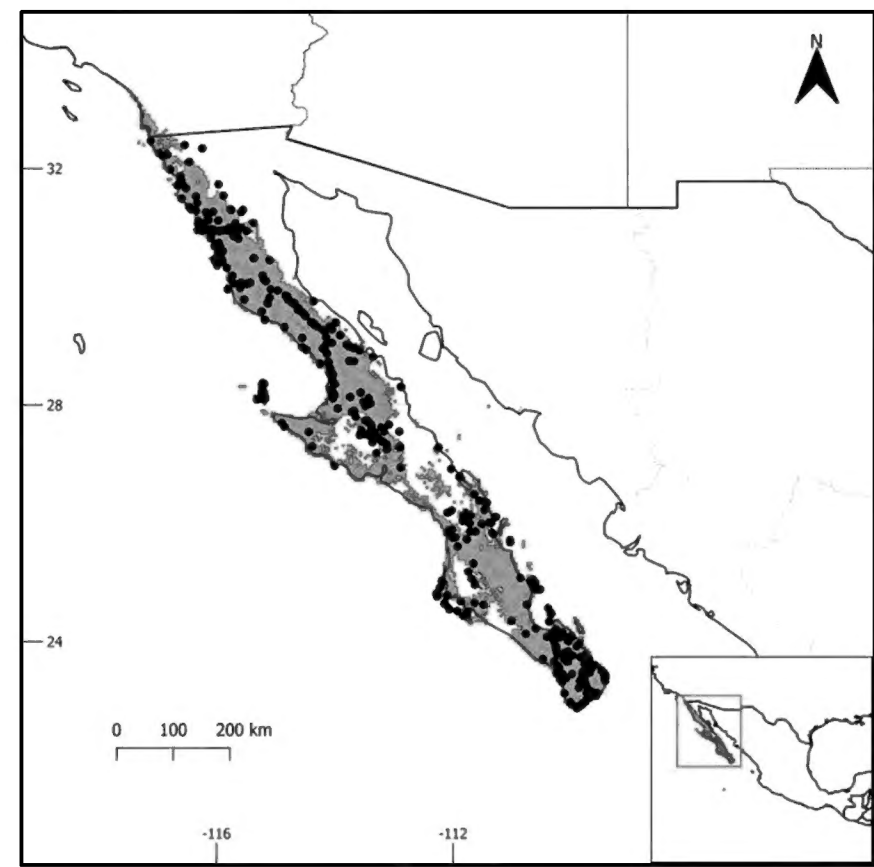
Sceloporus variabilis



Sceloporus virgatus



Sceloporus woodi



Sceloporus zosteromus